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ORIGINAL RESEARCH

Association between polymorphisms in microRNAs and ischemic stroke in an Asian population: evidence based on 6,083 cases and 7,248 controls

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Background: Polymorphisms in miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832) and miR-499 (rs3746444) have been associated with ischemic stroke (IS), but studies have given inconsistent results.

Methods: This meta-analysis investigated the possible association between IS risk and the four polymorphisms. A total of 14 case-control studies from Asian populations involving 6,083 cases and 7,248 controls for the four polymorphisms were included.

Results: Results showed that the GG genotype of miR-146a (rs2910164) may be associated with increased IS risk according to the recessive model (OR=1.20, 95% CI=1.02-1.42, *P*=0.03). Similarly, the CC genotype of miR-149 (rs2292832) may be associated with increased IS risk according to the recessive model (OR=1.28, 95% CI=1.08-1.52, *P*=0.005) and the homozygous model (OR=1.31, 95% CI=1.09-1.58, *P*=0.004). In contrast, miR-196a2 (rs11614913) and miR-499 (rs3746444) polymorphisms did not show significant association with IS risk in any of the five genetic models.

Conclusion: These results indicate that the GG genotype of miR-146a (rs2910164) and CC genotype of miR-149 (rs2292832) may confer increased susceptibility to IS, while miR-196a2 (rs11614913) and miR-499 (rs3746444) polymorphisms may not be associated with IS risk in Asian populations. These conclusions should be verified in large and well-designed studies.

Keywords: miRNAs, polymorphism, ischemic stroke, meta-analysis

Introduction

Stroke is a significant worldwide problem. An estimated 80% of the patients survive for at least 1 year after stroke, yet >70% have enduring disabilities.[1,2] Ischemic stroke (IS) and intracerebral hemorrhage account for ~80%–85% and 15%–20% of all stroke cases, respectively.[3] IS is a complex syndrome whose pathological development involves multiple components, which include environmental and genetic factors.[4] Established environmental risk factors include age, sex, body mass index, hypertension, diabetes mellitus, smoking, and hyperlipidemia. However, recent studies suggested that genetics may contribute more than environment to IS, considering that a number of single-gene disorders are related to IS.[5–8] Nevertheless, the factors defining genetic susceptibility to IS remain unclear.

MicroRNAs (miRNAs) represent a group of short non-coding RNA molecules, 18–25 nucleotides in length. Bioinformatics data indicate that a single miRNA can bind to as many as 200 gene targets, and miRNAs may regulate the expression of approximately one-third of protein-coding mRNAs. A single-nucleotide polymorphism

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(SNP) in miRNA may create a mismatch, leading to gene expression disorder and diseases.[9] Evidence has indicated that miRNAs regulate various IS-related biological processes, such as atherosclerosis, hypertension, and plaque rupture.[10] In fact, altered miRNA expression has been observed in IS in preclinical animal models and patients, suggesting a potential role in predicting the diagnosis and prognosis of IS.[11,12]

More specifically, the literature suggests an association between IS and polymorphisms in miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444).[13–26] However, these associations are controversial because individual studies relied on relatively small samples. Therefore, to obtain a more comprehensive understanding of the available evidence, we conducted this meta-analysis of 14 case–control studies to evaluate the possible association between IS risk and miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444) in Asian populations.

Materials and methods

Search strategy

All clinical and experimental case–control studies of miRNA polymorphisms and IS risk published through February 1, 2018 were identified through systematic searches in PubMed, EMBASE, Google Scholar, and the Chinese National Knowledge Infrastructure (CNKI) databases using English and Chinese. The search terms used were as follows: microRNA; miRNA; these two terms in combination with polymorphism, polymorphisms, SNP, variant, variants, variation, genotype, genetic, or mutation; and all the above-mentioned terms in combination with stroke or ischemic stroke. Reference lists in identified articles and reviews were also searched manually to identify additional eligible studies.

Inclusion criteria

To be included in our review and meta-analysis, studies had to 1) have a case–control design for assessing the association of IS risk with miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444); 2) be accessible as a full-text article and report sufficient data for estimating ORs with 95% CIs; 3) report genotype frequencies; and 4) involve humans rather than animal models.

Data extraction

Two authors (DHZ and CBL) independently extracted the following data from the included studies: first author's family name, year of publication, ethnicity, testing methods, control source, age, sex, *P*-value for Hardy–Weinberg equilibrium (HWE) in controls, numbers and genotypes of cases and controls, and frequencies of genotypes in cases and controls. Discrepancies were resolved by consensus. Only those studies that met the predetermined inclusion criteria were included.

Assessment of methodological quality

To assess the quality of the studies included in this analysis, the Newcastle–Ottawa scale was used by two independent assessors (JRW and LC).[27] For the Newcastle–Ottawa scale, a full score is nine stars; a score range of 5–9 stars is considered to indicate generally high methodological quality, whereas a range of 0–4 stars is considered to indicate poor quality.[28] The quality of all the included studies is summarized in Table 1. Any disagreements about Newcastle–Ottawa scores were resolved by other authors following a comprehensive reassessment. Only high-quality studies were included in our meta-analysis.

Statistical analyses

The unadjusted OR with 95% CI was used to assess the strength of the association of IS risk with miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444) based on genotype frequencies in cases and controls. The significance of pooled ORs was determined using the *Z*-test, with $P<0.05$ defined as the significance threshold. Meta-analysis was conducted using a fixed-effect model when $P>0.10$ for the *Q*-test, indicating the lack of heterogeneity among studies; otherwise, a random-effect model was used. All these statistical tests were performed using Review Manager 5.2 (Cochrane Collaboration, Oxford, England).

Publication bias was assessed using Begg's funnel plot and Egger's weighted regression, with $P<0.05$ considered statistically significant. Begg's funnel plots and Egger's weighted regression were calculated using Stata 12.0 (StataCorp LP, College Station, TX, USA).

Results

Description of studies

Figure 1 is a flow diagram illustrating the process of searching for and selecting studies. A total of 184 potentially relevant publications up to February 1, 2018 were systematically identified through searches of the PubMed, EMBASE, Google Scholar, and CNKI databases in English

Table 1 Methodological quality of the studies included in the final analysis based on the Newcastle–Ottawa scale for assessing the quality of case–control studies

Study	Selection (score)				Comparability (score)	Exposure (score)			Total score[b]
	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[a]	
Sun[13]	1	1	0	1	2	0	1	1	7
Li[14]	1	1	0	1	0	0	1	1	5
He and Han[15]	1	1	0	1	2	0	1	1	7
Jeon et al[16]	1	1	0	1	2	0	1	1	7
Hu et al[17]	1	1	0	1	2	0	1	1	7
Liu et al[18]	1	1	0	1	1	0	1	1	6
Zhu et al[19]	1	1	0	1	2	0	1	1	7
Huang et al[20]	1	1	0	1	2	0	1	1	7
Zhong et al[21]	1	1	0	1	2	0	1	1	7
Qu et al[22]	1	1	0	1	0	0	1	1	5
Lyu et al[23]	1	1	0	1	2	0	1	1	7
Zhu[24]	1	1	0	1	2	0	1	1	7
Luo et al[25]	1	1	0	1	2	0	1	1	7
Zhu et al[26]	1	1	0	1	2	0	1	0	6

Notes: [a]When there was no significant difference in the response rate between both groups based on a chi-squared test ($P>0.05$), one point was awarded. [b]Total score was calculated by adding up the points awarded in each item.

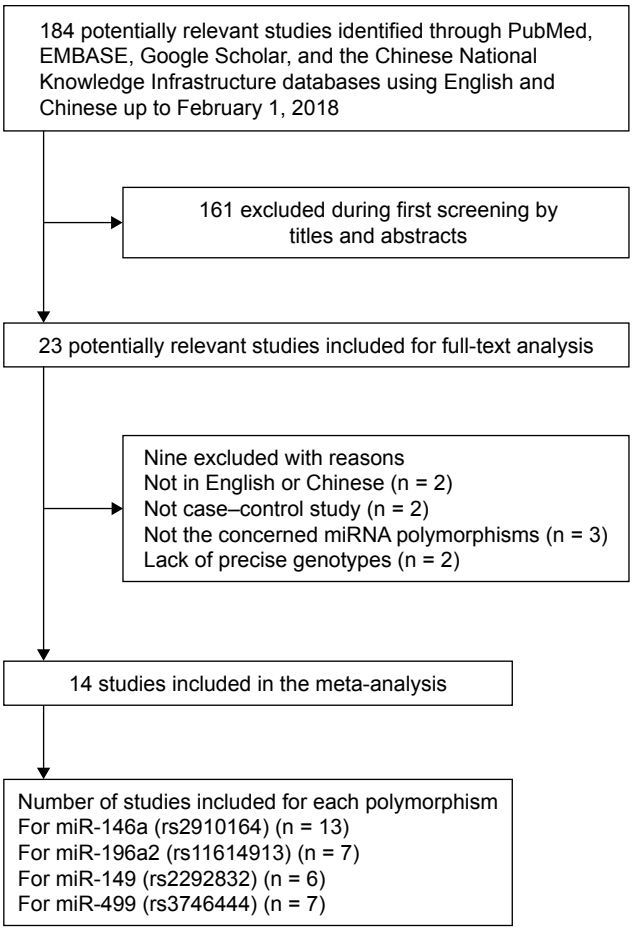


Figure 1 Flowchart of study selection.

and Chinese. Of these, we excluded 161 studies during initial screening based on review of the titles and abstracts. During analysis of the full text of the remaining articles, two studies were excluded for not being case–control studies, three studies were excluded because they did not report precise genotypes, and two articles were excluded because they investigated polymorphisms of miRNAs other than miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), or miR-499 (rs3746444). A further two studies were excluded because they were not written in English or Chinese.

In the end, 14 studies[13–26] were included in this meta-analysis based on our search strategy and inclusion criteria. Their characteristics are summarized in Table 2. Of these, 13 studies[13,14,16–26] (Table 3) involving 5,726 cases and 7,175 controls evaluated the association between miR-146a (rs2910164) polymorphism and IS risk. Seven studies[16,18–20,24–26] (Table 3) involving 3,090 cases and 3,047 controls evaluated the association between miR-196a2 (rs11614913) polymorphism and IS risk. Six studies[15–17,24–26] (Table 3) involving 2,448 cases and 2,322 controls evaluated miR-149 (rs2292832) polymorphism and IS risk. The remaining seven studies[16,18,20,23–26] (Table 3) involving 3,082 cases and 3,044 controls evaluated miR-499 (rs3746444) polymorphism and IS risk. The distribution of genotypes in controls was consistent with HWE ($P>0.05$) in all but three studies.[14,20,22] The overall quality of the included studies was adequate, and the mean Newcastle–Ottawa score for the included studies was 6.57 (Table 1).

Quantitative data synthesis

IS risk and miR-146a (rs2910164) polymorphism

The overall results for miR-146a (rs2910164) are summarized in Table 4 and Figure 2. On the basis of 5,726 cases and 7,175 controls from 13 studies,[13,14,16–26] the overall results indicated that the GG genotype of miR-146a (rs2910164) may be associated with increased IS risk according to the recessive model (OR=1.20, 95% CI=1.02–1.42, P=0.03; Figure 2B).

Table 2 Characteristics of the studies included in the meta-analysis

Study	Year	Ethnicity	Country	Testing method	Control source	Age (years, mean±SD)		Male, n (%)		SNP
						Cases	Controls	Cases	Controls	
Sun[13]	2011	Asian	China	PCR-RFLP	Hospital-based healthy volunteers	63±12	62±13	236 (61.9)	347 (53.4)	miR-146a (rs2910164)
Li[14]	2010	Asian	China	PCR-RFLP	Hospital-based healthy volunteers	64±11	45±12	188 (67.2)	579 (57.3)	miR-146a (rs2910164)
He and Han[15]	2013	Asian	China	PCR-RFLP	Hospital-based healthy volunteers	65.7±11.5	66.3±10.2	205 (55.0)	193 (51.7)	miR-149 (rs2292832)
Jeon et al[16]	2013	Asian	South Korea	TaqMan	Hospital-based healthy volunteers	64.16±11.90	63.14±10.19	336 (49.6)	244 (44.1)	miR-146a (rs2910164); miR-196a2 (rs11614913);

(*Continued*)

Table 2 (*Continued*)

Study	Year	Ethnicity	Country	Testing method	Control source	Age (years, mean±SD)		Male, n (%)		SNP
						Cases	Controls	Cases	Controls	
										miR-149 (rs2292832); and miR-499 (rs3746444)
Hu et al[17]	2014	Asian	China	PCR-RFLP	Hospital-based healthy volunteers	64±11.7	63±10.5	94 (48.0)	95 (46.3)	miR-146a (rs2910164) and miR-149 (rs2292832)
Liu et al[18]	2014	Asian	China	PCR-RFLP	Hospital-based healthy volunteers	67.52±10.29	66.34±11.07	227 (58.06)	180 (60.81)	miR-146a (rs2910164); miR-196a2 (rs11614913); and miR-499 (rs3746444)
Zhu et al[19]	2014	Asian	China	PCR-LDR	Hospital-based healthy volunteers	61.62±0.986	62.05±0.982	253 (68.75)	261 (68.50)	miR-146a (rs2910164) and miR-196a2 (rs11614913)
Huang et al[20]	2015	Asian	China	TaqMan	Hospital-based healthy volunteers	63 (54–70)[a]	61 (54–68)[a]	327 (61.6)	327 (61.6)	miR-146a (rs2910164); miR-196a2 (rs11614913); and miR-499 (rs3746444)
Zhong et al[21]	2016	Asian	China	PCR	Hospital-based healthy volunteers	62.6±8.63	61.1±9.58	177 (59.6)	170 (56.7)	miR-146a (rs2910164)
Qu et al[22]	2016	Asian	China	PCR-LDR	Hospital-based healthy volunteers	61.30±9.40	59.50±8.50	718 (63.0)	903 (57.0)	miR-146a (rs2910164)
Lyu et al[23]	2016	Asian	China	TaqMan	Hospital-based healthy volunteers	58±11.9	58±11.9	210 (55.6)	210 (55.6)	miR-146a (rs2910164) and miR-499 (rs3746444)
Zhu[24]	2016	Asian	China	PCR-RFLP	Hospital-based healthy volunteers	63.74±4.49	63.31±4.84	215 (54.3)	202 (53.4)	miR-146a (rs2910164); miR-196a2 (rs11614913); miR-149 (rs2292832); and miR-499 (rs3746444)
Luo et al[25]	2017	Asian	China	PCR	Hospital-based healthy volunteers	67.70±12.33	60.17±10.32	196 (65.8)	181 (59.8)	miR-146a (rs2910164); miR-196a2 (rs11614913); miR-149 (rs2292832); and miR-499 (rs3746444)
Zhu et al[26]	2017	Asian	China	TaqMan	Hospital-based healthy volunteers	61.0±10.2	59.7±9.9	321 (62.9)	311 (59.4)	miR-146a (rs2910164); miR-196a2 (rs11614913); miR-149 (rs2292832); and miR-499 (rs3746444)

Note: [a]These data are expressed as median (25th, 75th quartiles).
Abbreviations: LDR, ligase detection reaction; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single-nucleotide polymorphism.

Table 3 Genotype distributions of miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444)

Study	Year	*P*-value for HWE	Sample size (cases/controls)	No of cases			Allele frequencies of cases, n (%)		No of controls			Allele frequencies of controls, n (%)	
miR-146a (rs2910164)				**CC**	**GC**	**GG**	**C**	**G**	**CC**	**GC**	**GG**	**C**	**G**
Sun[13]	2011	0.345	358/650	136	161	61	433 (60.5)	283 (39.5)	228	304	118	760 (58.5)	540 (41.5)
Li[14]	2010	0.009	268/1,010	79	110	79	268 (50.0)	268 (50.0)	345	455	210	1,145 (56.7)	875 (43.3)
Jeon et al[16]	2013	0.589	678/553	223	327	128	773 (57.0)	583 (43.0)	211	266	76	688 (62.2)	418 (37.8)
Hu et al[17]	2014	0.193	196/205	75	87	34	237 (60.5)	155 (39.5)	97	82	26	276 (67.3)	134 (32.7)
Liu et al[18]	2014	0.650	296/391	85	159	52	329 (55.6)	263 (44.4)	116	198	77	430 (55.0)	352 (45.0)
Zhu et al[19]	2014	0.952	368/381	145	173	50	463 (63.0)	273 (37.0)	132	185	64	449 (80.6)	313 (19.4)
Huang et al[20]	2015	0.106	531/531	189	261	81	639 (60.2)	423 (39.8)	219	257	55	695 (65.4)	367 (34.6)
Zhong et al[21]	2016	0.133	297/300	141	128	28	410 (69.0)	184 (31.0)	113	152	35	378 (63.0)	222 (37.0)
Qu et al[22]	2016	<0.001	1,139/1,585	355	618	166	1,328 (58.3)	950 (41.7)	483	869	233	1,835 (57.9)	1,335 (42.1)
Lyu et al[23]	2016	0.079	378/378	119	198	61	436 (57.7)	320 (42.3)	153	187	38	493 (65.2)	263 (34.8)
Zhu[24]	2016	0.521	396/378	131	194	71	456 (57.6)	336 (42.4)	154	179	45	487 (64.4)	269 (35.6)
Luo et al[25]	2017	0.672	298/303	129	130	39	388 (65.1)	208 (34.9)	119	139	45	377 (62.2)	229 (37.8)
Zhu et al[26]	2017	0.085	523/510	170	267	86	607 (58.0)	439 (42.0)	204	251	55	659 (64.6)	361 (35.4)
miR-196a2 (rs11614913)				**TT**	**TC**	**CC**	**T**	**C**	**TT**	**TC**	**CC**	**T**	**C**
Jeon et al[16]	2013	0.126	678/553	139	352	187	630 (46.5)	726 (53.5)	105	292	156	502 (45.4)	604 (54.6)
Liu et al[18]	2014	0.060	296/391	51	181	64	283 (47.8)	309 (52.2)	84	214	93	382 (48.8)	400 (51.2)
Zhu et al[19]	2014	0.384	368/381	71	189	108	331 (45.0)	405 (55.0)	78	198	105	354 (46.5)	408 (53.5)
Huang et al[20]	2015	0.856	531/531	100	265	166	465 (43.8)	597 (56.2)	112	266	153	490 (46.1)	572 (53.9)
Zhu[24]	2016	0.354	396/378	112	205	79	429 (54.2)	363 (45.8)	110	196	72	416 (55.0)	340 (45.0)
Luo et al[25]	2017	0.385	298/303	73	138	87	284 (47.7)	312 (52.3)	75	159	69	309 (51.0)	297 (49.0)
Zhu et al[26]	2017	0.548	523/510	150	273	100	573 (54.8)	473 (45.2)	146	260	104	552 (54.1)	468 (45.9)
miR-149 (rs2292832)				**TT**	**TC**	**CC**	**T**	**C**	**TT**	**TC**	**CC**	**T**	**C**
He and Han[15]	2013	0.303	357/373	138	162	57	438 (66.6)	276 (41.4)	160	175	38	495 (66.4)	251 (33.6)
Jeon et al[16]	2013	0.921	678/553	299	303	76	901 (66.4)	455 (33.6)	262	238	53	762 (68.9)	344 (31.1)
Hu et al[17]	2014	0.199	196/205	79	76	41	234 (59.7)	158 (40.3)	80	89	36	249 (60.7)	161 (39.3)
Zhu[24]	2016	0.720	396/378	165	179	52	509 (64.3)	283 (35.7)	190	158	30	538 (71.2)	218 (28.8)
Luo et al[25]	2017	0.447	298/303	131	127	40	389 (65.3)	207 (34.7)	121	136	46	378 (62.4)	228 (37.6)
Zhu et al[26]	2017	0.351	523/510	232	221	70	685 (65.5)	361 (34.5)	240	213	57	693 (67.9)	327 (32.1)
miR-499 (rs3746444)				**AA**	**AG**	**GG**	**A**	**G**	**AA**	**AG**	**GG**	**A**	**G**
Jeon et al[16]	2013	0.740	678/553	460	195	23	1,115 (82.2)	241 (17.8)	365	170	18	900 (81.4)	206 (18.6)
Liu et al[18]	2014	0.170	296/391	181	96	19	458 (77.4)	134 (22.6)	278	99	14	655 (83.8)	127 (16.2)
Huang et al[20]	2015	0.002	531/531	398	133	0	929 (87.5)	133 (12.5)	403	128	0	934 (87.9)	128 (12.1)
Lyu et al[23]	2016	0.621	378/378	257	110	11	624 (82.5)	132 (17.5)	250	113	15	613 (81.1)	143 (18.9)
Zhu[24]	2016	0.910	396/378	255	123	18	633 (79.9)	159 (20.1)	249	116	13	614 (81.2)	142 (18.8)
Luo et al[25]	2017	0.131	298/303	215	78	5	508 (85.2)	88 (14.8)	244	53	6	541 (89.3)	65 (10.7)
Zhu et al[26]	2017	0.380	505/510	349	124	32	840 (80.3)	206 (19.7)	328	158	24	814 (79.8)	206 (20.2)

Abbreviation: HWE, Hardy–Weinberg equilibrium.

Table 4 Overall meta-analysis of the association between ischemic stroke and polymorphisms in miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444)

Genetic model	OR [95% CI]	Z (*P*-value)	Heterogeneity of study design			Analysis model
			χ^2	*df* (*P*-value)	I^2 (%)	
miR-146a (rs2910164) from 13 case–control studies (5,726 cases and 7,175 controls)						
Allelic model (G-allele vs C-allele)	1.10 [0.99–1.22]	1.74 (0.08)	47.91	12 (<0.001)	75	Random
Recessive model (GG vs GC+CC)	1.20 [1.02–1.42]	2.16 (0.03)	31.55	12 (0.002)	62	Random
Dominant model (CC vs GC+GG)	0.91 [0.80–1.04]	1.41 (0.16)	34.76	12 (<0.001)	65	Random
Homozygous model (GG vs CC)	1.24 [1.00–1.53]	1.95 (0.05)	43.43	12 (<0.001)	72	Random
Heterozygous model (GC vs CC)	1.06 [0.95–1.17]	1.00 (0.32)	20.79	12 (0.05)	42	Random

(*Continued*)

Table 4 (*Continued*)

Genetic model	OR [95% CI]	Z (*P*-value)	Heterogeneity of study design			Analysis model
			??2	*df* (*P*-value)	I^2 (%)	
miR-196a2 (rs11614913) from 7 case–control studies (3,090 cases and 3,047 controls)						
Allelic model (C-allele vs T-allele)	1.04 [0.97–1.12]	1.10 (0.27)	3.20	6 (0.78)	0	Fixed
Recessive model (CC vs TC+TT)	1.04 [0.93–1.17]	0.73 (0.46)	4.60	6 (0.60)	0	Fixed
Dominant model (TT vs TC+CC)	0.95 [0.85–1.08]	0.77 (0.44)	2.86	6 (0.83)	0	Fixed
Homozygous model (CC vs TT)	1.07 [0.92–1.24]	0.91 (0.36)	2.85	6 (0.83)	0	Fixed
Heterozygous model (TC vs TT)	1.07 [0.93–1.23]	0.90 (0.37)	2.72	5 (0.74)	0	Fixed
miR-149 (rs2292832) from 6 case–control studies (2,448 cases and 2,322 controls)						
Allelic model (C-allele vs T-allele)	1.09 [1.00–1.18]	1.91 (0.06)	4.84	5 (0.44)	0	Fixed
Recessive model (CC vs TC+TT)	1.28 [1.08–1.52]	2.80 (0.005)	6.14	5 (0.29)	19	Fixed
Dominant model (TT vs TC+CC)	0.89 [0.79–1.00]	1.99 (0.05)	6.31	5 (0.28)	21	Fixed
Homozygous model (CC vs TT)	1,31 [1.09–1.58]	2.92 (0.004)	8.27	5 (0.14)	40	Fixed
Heterozygous model (TC vs TT)	1.07 [0.95–1.21]	1.12 (0.26)	4.22	5 (0.52)	0	Fixed
miR-499 (rs3746444) from 7 case–control studies (3,082 cases and 3,044 controls)						
Allelic model (G-allele vs A-allele)	1.09 [0.95–1.25]	1.28 (0.20)	12.36	6 (0.05)	51	Random
Recessive model (GG vs AG+AA)	1.21 [0.91–1.61]	1.31 (0.19)	3.81	5 (0.58)	0	Fixed
Dominant model (AA vs AG+GG)	0.93 [0.78–1.12]	0.77 (0.44)	16.43	6 (0.01)	63	Random
Homozygous model (GG vs AA)	1.20 [0.90–1.60]	1.25 (0.21)	4.47	5 (0.48)	0	Fixed
Heterozygous model (AG vs AA)	1.06 [0.87–1.28]	0.56 (0.57)	17.10	6 (0.009)	65	Random

IS risk and miR-196a2 (rs11614913) polymorphism

The overall results are summarized in Table 4 and Figure 3. On the basis of 3,090 cases and 3,047 controls from seven studies,[16,18–20,24–26] miR-196a2 (rs11614913) polymorphism did not show significant association with IS risk in any of the following five genetic models: allelic model, OR=1.04, 95% CI=0.97–1.12, *P*=0.27 (Figure 3A); recessive model, OR=1.04, 95% CI=0.93–1.17, *P*=0.46 (Figure 3B); dominant model, OR=0.95, 95% CI=0.85–1.08, *P*=0.44 (Figure 3C); homozygous model, OR=0.95, 95% CI=0.85–1.08, *P*=0.44 (Figure 3D); and heterozygous model, OR=1.07, 95% CI=0.93–1.23, *P*=0.37 (Figure 3E).

IS risk and miR-149 (rs2292832) polymorphism

The overall results for miR-149 (rs2292832) are summarized in Table 4 and Figure 4. On the basis of 2,448 cases and

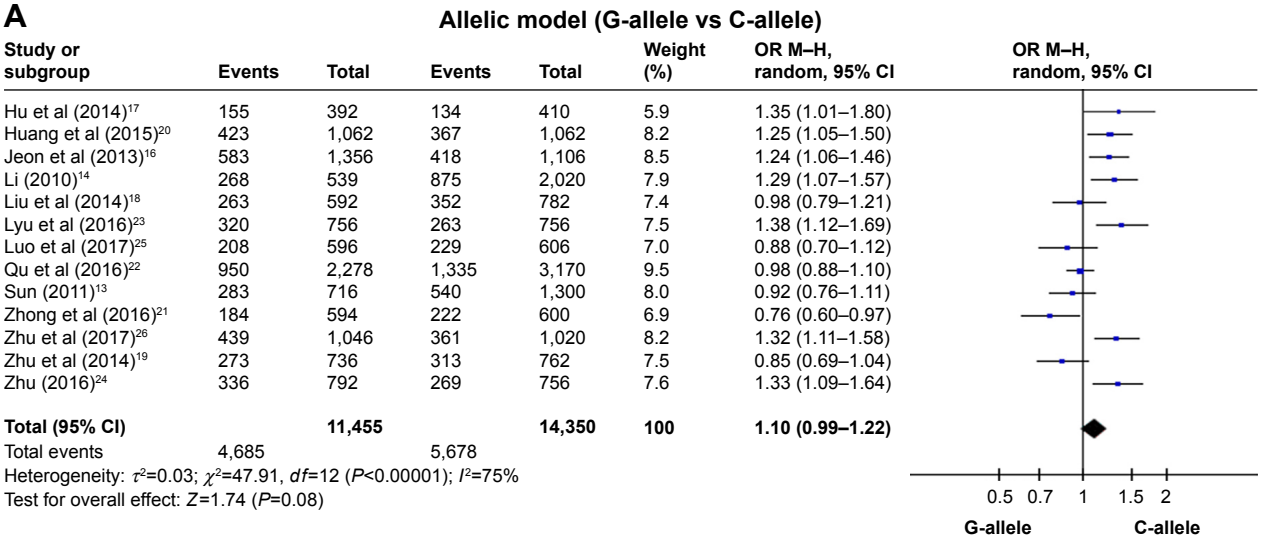


Figure 2 (*Continued*)

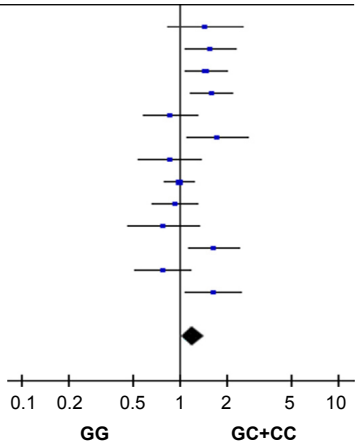
B

Recessive model (GG vs GC+CC)

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, random, 95% CI
Hu et al (2014)[17]	34	196	26	205	5.3	1.44 (0.83–2.51)
Huang et al (2015)[20]	81	531	55	531	8.0	1.56 (1.08–2.25)
Jeon et al (2013)[16]	128	678	76	553	9.0	1.46 (1.07–1.99)
Li (2010)[14]	79	268	210	1,010	9.1	1.59 (1.18–2.16)
Liu et al (2014)[18]	52	296	77	391	7.6	0.87 (0.59–1.28)
Lyu et al (2016)[23]	61	378	38	378	6.9	1.72 (1.12–2.85)
Luo et al (2017)[25]	39	298	45	303	6.5	0.86 (0.54–1.37)
Qu et al (2016)[22]	166	1,139	233	1,585	10.7	0.99 (0.80–1.23)
Sun (2011)[13]	61	358	118	650	8.4	0.93 (0.66–1.30)
Zhong et al (2016)[21]	28	297	35	300	5.7	0.79 (0.47–1.33)
Zhu et al (2017)[26]	86	523	55	510	8.0	1.63 (1.13–2.34)
Zhu et al (2014)[19]	50	368	64	381	7.4	0.78 (0.52–1.16)
Zhu (2016)[24]	71	396	45	378	7.4	1.62 (1.08–2.42)
Total (95% CI)		**5,726**		**7,175**	**100**	**1.20 (1.02–1.42)**
Total events	936		1,077			

Heterogeneity: τ^2=0.06; χ^2=31.55, *df*=12 (*P*=0.002); I^2=62%

Test for overall effect: *Z*=2.16 (*P*=0.03)



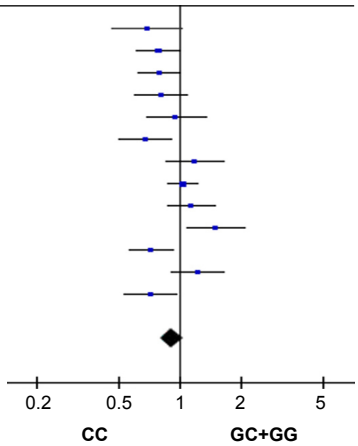
C

Dominant model (CC vs GC+GG)

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, random, 95% CI
Hu et al (2014)[17]	75	196	97	205	5.7	0.69 (0.46–1.03)
Huang et al (2015)[20]	189	531	219	531	8.5	0.79 (0.61–1.01)
Jeon et al (2013)[16]	223	678	211	553	8.7	0.79 (0.63–1.00)
Li (2010)[14]	79	268	345	1,010	7.5	0.81 (0.60–1.08)
Liu et al (2014)[18]	85	296	116	391	6.8	0.96 (0.68–1.33)
Lyu et al (2016)[23]	119	378	153	378	7.4	0.68 (0.50–0.91)
Luo et al (2017)[25]	129	298	119	303	6.9	1.18 (0.85–1.63)
Qu et al (2016)[22]	355	1,139	483	1,585	10.2	1.03 (0.88–1.22)
Sun (2011)[13]	136	358	228	650	8.0	1.13 (0.87–1.48)
Zhong et al (2016)[21]	141	297	113	300	6.9	1.50 (1.08–2.07)
Zhu et al (2017)[26]	170	523	204	510	8.3	0.72 (0.56–0.93)
Zhu et al (2014)[19]	145	368	132	381	7.5	1.23 (0.91–1.65)
Zhu (2016)[24]	131	396	154	378	7.5	0.72 (0.54–0.96)
Total (95% CI)		**5,726**		**7,715**	**100**	**0.91 (0.80–1.04)**
Total events	1,977		2,574			

Heterogeneity: τ^2=0.04; χ^2=34.76, *df*=12 (*P*=0.0005); I^2=65%

Test for overall effect: *Z*=1.41 (*P*=0.16)



D

Homozygous model (GG vs CC)

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, random, 95% CI
Hu et al (2014)[17]	34	109	26	123	6.0	1.69 (0.93–3.06)
Huang et al (2015)[20]	81	270	55	274	8.0	1.71 (1.15–2.53)
Jeon et al (2013)[16]	128	351	76	283	8.6	1.56 (1.11–2.20)
Li (2010)[14]	79	158	210	555	8.5	1.64 (1.15–2.35)
Liu et al (2014)[18]	52	137	77	193	7.4	0.92 (0.59–1.44)
Lyu et al (2016)[23]	61	180	38	191	7.2	2.06 (1.29–3.30)
Luo et al (2017)[25]	39	168	45	164	6.9	0.80 (0.49–1.31)
Qu et al (2016)[22]	166	521	233	716	9.7	0.97 (0.76–1.23)
Sun (2011)[13]	61	197	118	346	8.2	0.87 (0.60–1.26)
Zhong et al (2016)[21]	28	169	35	148	6.3	0.64 (0.37–1.12)
Zhu et al (2017)[26]	86	256	55	259	8.0	1.88 (1.26–2.78)
Zhu et al (2014)[19]	50	195	64	196	7.5	0.71 (0.46–1.10)
Zhu (2016)[24]	71	202	45	199	7.5	1.85 (1.19–2.88)
Total (95% CI)		**2,913**		**3,647**	**100**	**1.24 (1.00–1.53)**
Total events	936		1,077			

Heterogeneity: τ^2=0.11; χ^2=43.43, *df*=12 (*P*<0.0001); I^2=72%

Test for overall effect: *Z*=1.95 (*P*=0.05)

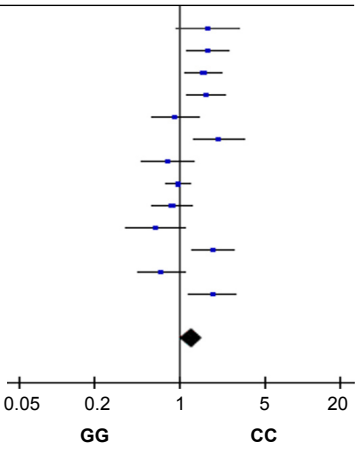


Figure 2 (*Continued*)

E Heterozygous model (GC vs CC)

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, random, 95% CI
Hu et al (2014)[17]	87	162	82	179	4.7	1.37 (0.90–2.10)
Huang et al (2015)[20]	261	450	257	476	8.9	1.18 (0.91–1.53)
Jeon et al (2013)[16]	327	550	266	477	9.3	1.16 (0.91–1.49)
Li (2010)[14]	110	189	455	800	7.0	1.06 (0.77–1.46)
Liu et al (2014)[18]	159	244	198	314	6.2	1.10 (0.77–1.55)
Lyu et al (2016)[23]	198	317	187	340	7.2	1.36 (1.00–1.86)
Luo et al (2017)[25]	130	259	139	258	6.3	0.86 (0.61–1.22)
Qu et al (2016)[22]	618	973	869	1,352	12.7	0.97 (0.82–1.15)
Sun (2011)[13]	161	297	304	532	8.0	0.89 (0.67–1.18)
Zhong et al (2016)[21]	128	269	152	265	6.4	0.67 (0.48–0.95)
Zhu et al (2017)[26]	267	437	251	455	8.7	1.28 (0.98–1.67)
Zhu et al (2014)[19]	173	318	185	317	7.2	0.85 (0.62–1.1 7)
Zhu (2016)[24]	194	325	179	333	7.3	1.27 (0.94–1.74)
Total (95% CI)		**4,790**		**6,098**	**100**	**1.06 (0.95–1.17)**
Total events	2,813		3,524			

Heterogeneity: τ^2=0.02; χ^2=20.79, *df*=12 (*P*=0.05); I^2=42%
Test for overall effect: *Z*=1.00 (*P*=0.32)

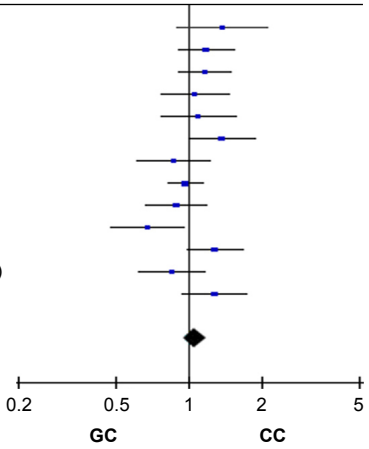
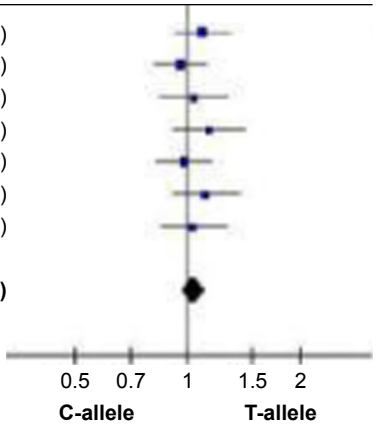


Figure 2 Forest plot describing the association between the miR-146a (rs2910164) polymorphism and ischemic stroke risk according to different genetic models: (**A**) allelic (G-allele vs C-allele), (**B**) recessive (GG vs GC+CC), (**C**) dominant (CC vs GC+GG), (**D**) homozygous (GG vs CC), and (**E**) heterozygous (GC vs CC).

A Allelic model (C-allele vs T-allele)

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, fixed, 95% CI
Huang et al (2015)[20]	597	1,062	572	1,062	16.8	1.10 (0.93–1.30)
Jeon et al (2013)[16]	726	1,356	604	1,106	20.7	0.96 (0.82–1.12)
Liu et al (2014)[18]	309	592	400	782	11.1	1.04 (0.84–1.29)
Luo et al (2017)[25]	312	596	297	606	9.4	1.14 (0.91–1.43)
Zhu et al (2017)[26]	413	1,046	468	1,020	17.4	0.97 (0.82–1.16)
Zhu et al (2014)[19]	405	736	408	782	11.9	1.12 (0.92–1.37)
Zhu (2016)[24]	363	792	340	756	12.6	1.04 (0.85–1.26)
Total (95% CI)		**6,180**		**6,114**	**100**	**1.04 (0.97–1.12)**
Total events	3,185		3,069			

Heterogeneity: χ^2=3.20, *df*=6 (*P*=0.78); I^2=0%
Test for overall effect: *Z*=1.10 (*P*=0.27)



B Recessive model (CC vs TC+TT)

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, fixed, 95% CI
Huang et al (2015)[20]	166	531	153	531	18.9	1.12 (0.86–1.46)
Jeon et al (2013)[16]	187	678	156	553	22.3	0.97 (0.75–1.24)
Liu et al (2014)[18]	64	296	93	391	11.3	0.88 (0.62–1.27)
Luo et al (2017)[25]	87	298	69	303	8.7	1.40 (0.97–2.02)
Zhu et al (2017)[26]	100	523	104	510	15.3	0.92 (0.68–1.25)
Zhu et al (2014)[19]	108	368	105	381	13.1	1.09 (0.79–1.50)
Zhu (2016)[24]	79	396	72	378	10.6	1.06 (0.74–1.51)
Total (95% CI)		**3,090**		**3,047**	**100**	**1.04 (0.93–1.17)**
Total events	791		752			

Heterogeneity: χ^2=4.60, *df*=6 (*P*=0.60); I^2=0%
Test for overall effect: *Z*=0.73 (*P*=0.46)

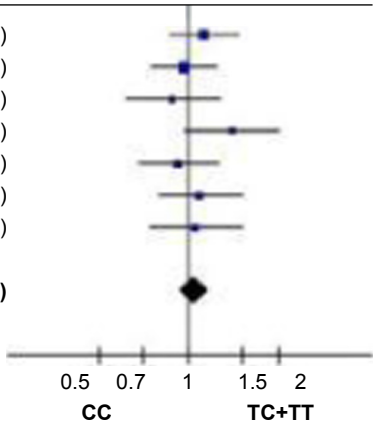


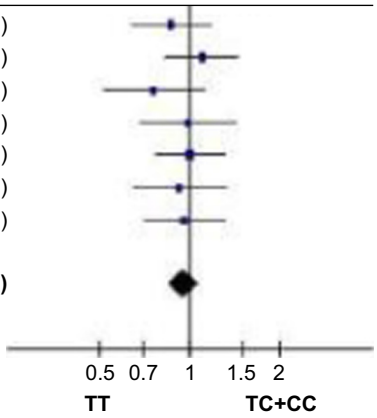
Figure 3 (*Continued*)

C

Dominant model (TT vs TC+CC)

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, fixed, 95% CI
Huang et al (2015)[20]	100	531	112	531	16.6	0.37 (0.64–1.17)
Jeon et al (2013)[16]	139	678	105	553	16.8	1.10 (0.83–1.46)
Liu et al (2014)[18]	51	296	84	391	11.0	0.76 (0.52–1.12)
Luo et al (2017)[25]	73	298	75	303	10.3	0.99 (0.68–1.43)
Zhu et al (2017)[26]	150	523	146	510	19.3	1.00 (0.77–1.31)
Zhu et al (2014)[19]	71	368	78	381	11.3	0.93 (0.65–1.33)
Zhu (2016)[24]	112	396	110	378	14.8	0.96 (0.70–1.31)
Total (95% CI)		**3,090**		**3,047**	**100**	**0.96 (0.85–1.08)**
Total events	696		710			

Heterogeneity: χ^2=2.86, *df*=6 (*P*=0.83); I^2=0%
Test for overall effect: *Z*=0.77 (*P*=0.44)

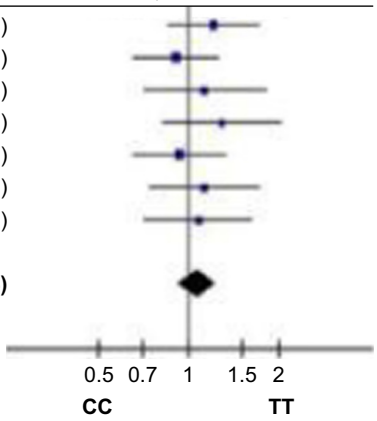


D

Homozygous model (CC vs TT)

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, fixed, 95% CI
Huang et al (2015)[20]	166	266	153	265	16.8	1.22 (0.86–1.72)
Jeon et al (2013)[16]	187	326	156	261	21.5	0.91 (0.65–1.26)
Liu et al (2014)[18]	64	115	93	177	9.4	1.13 (0.71–1.82)
Luo et al (2017)[25]	87	160	69	144	9.6	1.30 (0.82–2.03)
Zhu et al (2017)[26]	100	250	104	250	18.1	0.94 (0.66–1.34)
Zhu et al (2014)[19]	108	179	105	183	12.0	1.13 (0.74–1.72)
Zhu (2016)[24]	79	191	72	182	12.6	1.08 (0.71–1.63)
Total (95% CI)		**1,487**		**1,462**	**100**	**1.07 (0.92–1.24)**
Total events	791		752			

Heterogeneity: χ^2=2.85, *df*=6 (*P*=0.83); I^2=0%
Test for overall effect: *Z*=0.91 (*P*=0.36)



E

Heterozygous model (TC vs TT)

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, fixed, 95% CI
Huang et al (2015)[20]	265	365	266	378	18.7	1.12 (0.81–1.53)
Jeon et al (2013)[16]	352	291	292	397		Not estimable
Liu et al (2014)[18]	181	232	214	298	10.8	1.39 (0.93–2.08)
Luo et al (2017)[25]	138	211	159	234	13.7	0.89 (0.60–1.32)
Zhu et al (2017)[26]	273	423	260	406	24.6	1.02 (0.77–1.36)
Zhu et al (2014)[19]	189	260	198	275	13.7	1.05 (0.72–1.53)
Zhu (2016)[24]	205	317	196	306	18.4	1.03 (0.74–1.43)
Total (95% CI)		**2,099**		**2,295**	**100**	**1.07 (0.93–1.23)**
Total events	1,603		1,585			

Heterogeneity: χ^2=2.72, *df*=6 (*P*=0.74); I^2=0%
Test for overall effect: *Z*=0.90 (*P*=0.37)

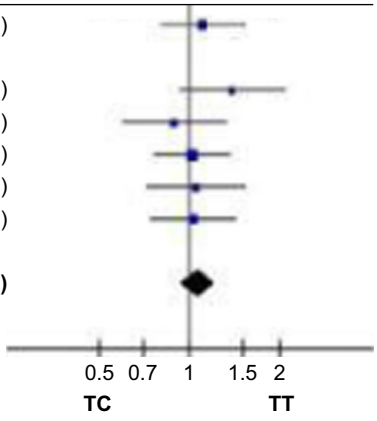


Figure 3 Forest plot describing the association between the miR-196a2 (rs11614913) polymorphism and ischemic stroke risk according to different genetic models: (**A**) allelic, (**B**) recessive, (**C**) dominant, (**D**) homozygous, and (**E**) heterozygous.

2,322 controls from six studies,[16,18,20,23–26] the overall results indicated that the CC genotype of miR-149 (rs2292832) may be associated with increased IS risk according to the recessive model (OR=1.28, 95% CI=1.08–1.52, *P*=0.005; Figure 4B) and homozygous model (OR=1.31, 95% CI=1.09–1.58, *P*=0.004; Figure 4D).

IS risk and miR-499 (rs3746444) polymorphism

The overall results are summarized in Table 4 and Figure 5. On the basis of 3,082 cases and 3,044 controls from seven studies,[16,18,20,23–26] miR-499 (rs3746444) polymorphism did not show significant association with IS risk in any of the following five genetic models: allelic model, OR=1.09, 95% CI=0.95–1.25, *P*=0.20 (Figure 5A); recessive model, OR=1.21, 95% CI=0.91–1.61, *P*=0.19 (Figure 5B); dominant model, OR=0.93, 95% CI=0.78–1.12, *P*=0.44 (Figure 5C); homozygous model, OR=1.20, 95% CI=0.90–1.60, *P*=0.21 (Figure 5D); or heterozygous model, OR=1.06, 95% CI=0.87–1.28, *P*=0.57 (Figure 5E).

Sensitivity analysis

Sensitivity analysis was conducted for miR-146a (rs2910164) by excluding the studies by Li et al[14] and Qu et al;[22] the *P*-value for HWE was less than 0.05 for these two studies. The recessive model gave different results (OR=1.19, 95% CI=0.98–1.45, *P*=0.07) than those obtained when all studies were meta-analyzed. Sensitivity analysis was conducted for miR-146a (rs2910164) by excluding one study by Jeon et al.[16] Again, the recessive model gave different results (OR=1.18, 95% CI=0.99–1.41, *P*=0.07) than when all studies were included. Therefore, the results for miR-146a (rs2910164) should be interpreted with caution.

Sensitivity analysis was conducted for miR-196a2 (rs11614913) by excluding the study by Jeon et al.[16] The results were similar to those obtained with all studies,

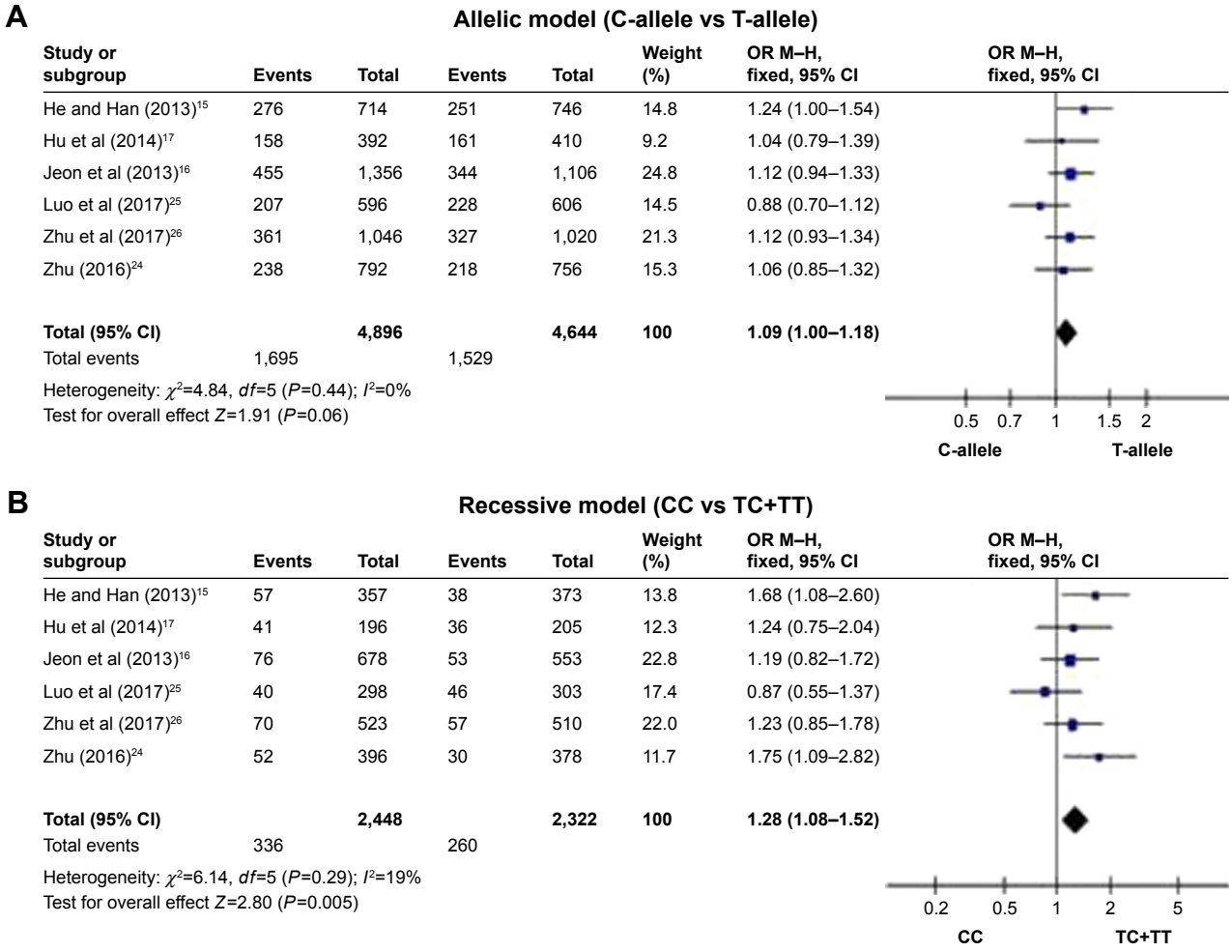


Figure 4 (*Continued*)

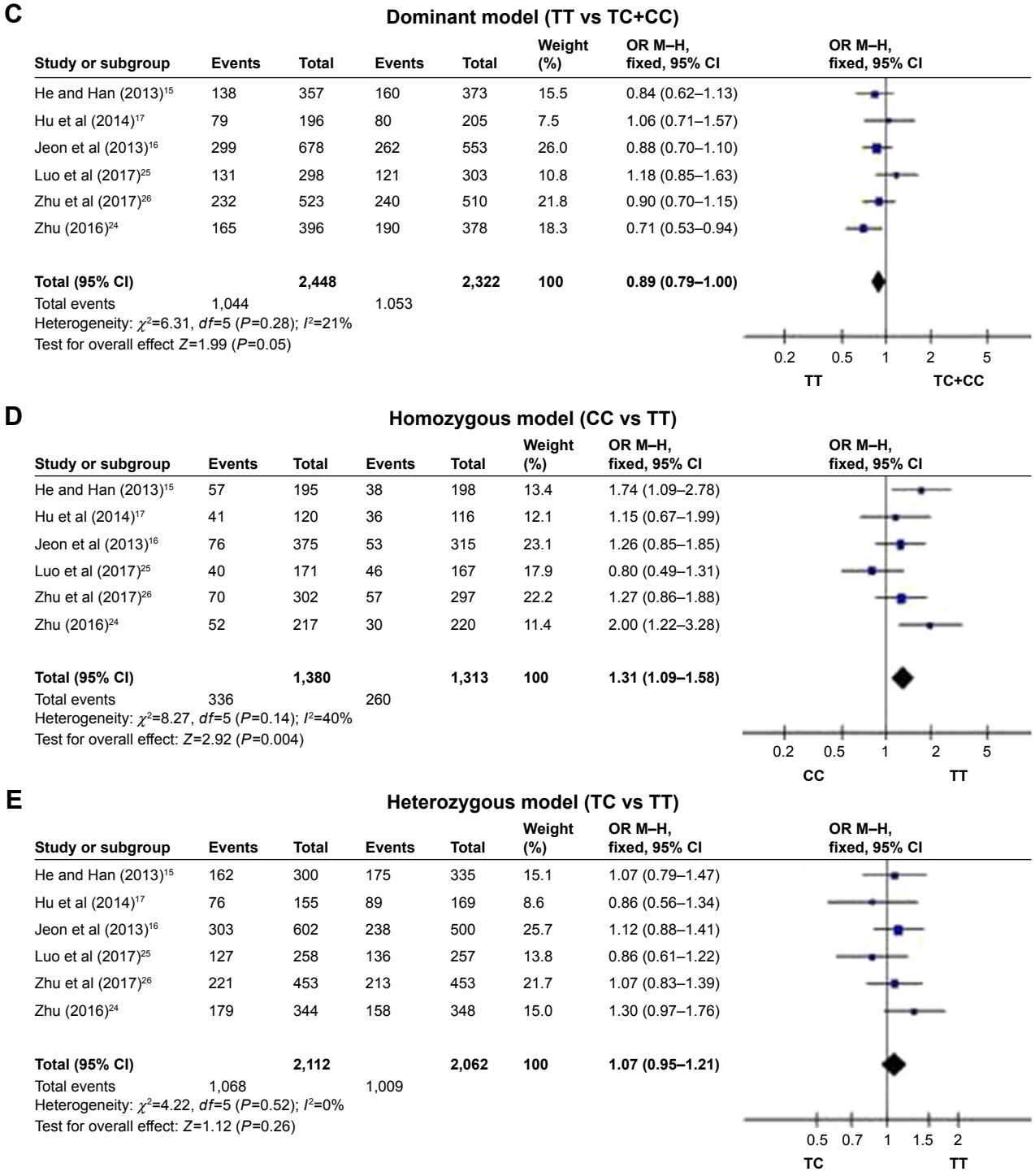


Figure 4 Forest plot describing the association between the miR-149 (rs2292832) polymorphism and ischemic stroke risk according to different genetic models: (**A**) allelic, (**B**) recessive, (**C**) dominant, (**D**) homozygous, and (**E**) heterozygous.

regardless of the genetic model. This implies that our meta-analysis results for miR-196a2 (rs11614913) are robust. Similar robustness was observed when we performed sensitivity analysis for miR-149 (rs2292832) and for miR-499 (rs3746444) by excluding the study by Jeon et al.[16]

Sensitivity analysis was conducted for miR-499 (rs3746444) by excluding a study by Huang et al,[20] in which the *P*-value of HWE was less than 0.05. The results were not altered in any of the five genetic models.

Publication bias

Begg's funnel plot and Egger's test were performed to detect potential publication bias in this meta-analysis. No obvious asymmetry was observed in Begg's funnel plots in

the recessive model, and Egger's tests (Figure 6) indicated no publication bias.

Discussion

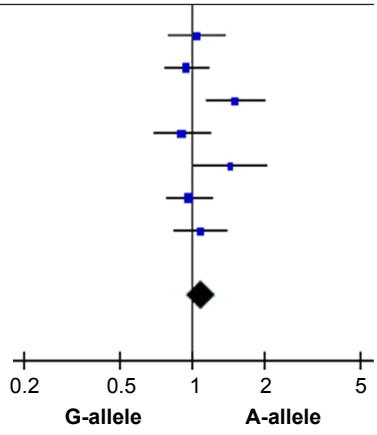
Previous studies have demonstrated that mutations in the pre-miRNA of miR-146a, miR-499, miR-149, and miR-196a2 decrease the levels of the corresponding mature miRNAs.[20,29,30] These four miRNAs affect thrombosis or inflammation pathways in the circulatory system by regulating tumor necrosis factor-α (TNF-α),[31] methylenetetrahydrofolate reductase,[32] annexin A1,[33] C-reactive protein,[34] the NF-κB pathway, and the MAP kinase pathway.[35] Many studies have been conducted to reveal the impact of SNPs on precursor and mature miRNAs and their associations with IS risk.[13–26] In fact, several meta-analyses have been conducted to explore the association between miRNA polymorphisms and IS risk. The results have been inconsistent, largely due to limited sample size.[36–39] Therefore, we conducted this meta-analysis on all eligible studies to provide a more precise estimate of the association of IS risk with miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444). Interestingly, all the case–control studies in our meta-analysis analyzed Asian populations.

A previous meta-analysis by Zhu et al[39] found the C allele of miR-146a (rs2910164) to be associated with lower IS risk, but this trend was observed only in Koreans according to the allelic model. Our meta-analysis, in contrast, suggests that this C allele is not significantly associated with IS risk; instead, we found the GG genotype of miR-146a (rs2910164) to be associated with increased risk. Our result may be more reliable than that of the previous meta-analysis by Zhu et al[39] because our meta-analysis contained nine more case–control studies[14,15,17,21–26] with larger samples. Our subgroup analysis suggesting a significant relationship

A **Allelic model (G-allele vs A-allele)**

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, random, 95% CI
Huang et al (2015)[20]	133	1,062	128	1,062	14.1	1.04 (0.81–1.35)
Jeon et al (2013)[16]	241	1,356	206	1,106	17.1	0.94 (0.77–1.16)
Liu et al (2014)[18]	134	592	127	782	13.5	1.51 (1.15–1.98)
Lyu et al (2016)[23]	132	756	143	756	14.0	0.91 (0.70–1.18)
Luo et al (2017)[25]	88	596	65	606	10.4	1.44 (1.02–2.03)
Zhu et al (2017)[26]	206	1,046	206	1,020	16.5	0.97 (0.78–1.20)
Zhu (2016)[24]	159	792	142	756	14.5	1.09 (0.84–1.40)
Total (95% CI)		**6,200**		**6,088**	**100**	**1.09 (0.94–1.25)**
Total events	1,093		1,017			

Heterogeneity: τ^2=0.02; χ^2=12.92, *df*=6 (*P*=0.04); I^2=54%
Test for overall effect: *Z*=1.16 (*P*=0.25)



B **Recessive model (GG vs AG+AA)**

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, fixed, 95% CI
Huang et al (2015)[20]	0	531	0	531		Not estimable
Jeon et al (2013)[16]	23	678	18	553	22.3	1.04 (0.56–1.95)
Liu et al (2014)[18]	19	296	14	391	13.1	1.85 (0.91–3.75)
Lyu et al (2016)[23]	11	378	15	378	16.9	0.73 (0.33–1.60)
Luo et al (2017)[25]	5	298	6	303	6.8	0.84 (0.25–2.80)
Zhu et al (2017)[26]	32	505	24	510	26.0	1.37 (0.80–2.36)
Zhu (2016)[24]	18	396	13	378	14.8	1.34 (0.65–2.77)
Total (95% CI)		**3,082**		**3,044**	**100**	**1.21 (0.91–1.61)**
Total events	108		90			

Heterogeneity: χ^2=3.81, *df*=5 (*P*=0.58); I^2=0%
Test for overall effect: *Z*=1.31 (*P*=0.19)

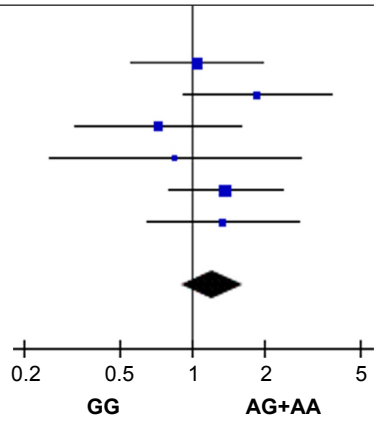
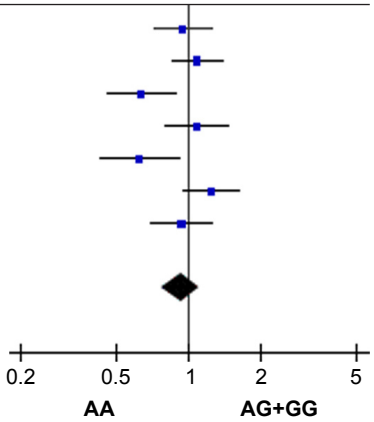


Figure 5 (*Continued*)

C Dominant model (AA vs AG+GG)

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, random, 95% CI
Huang et al (2015)[20]	398	531	403	531	14.9	0.95 (0.72–1.26)
Jeon et al (2013)[16]	460	678	365	553	16.4	1.09 (0.86–1.38)
Liu et al (2014)[18]	181	296	278	391	13.4	0.64 (0.46–0.88)
Lyu et al (2016)[23]	257	378	250	378	14.0	1.09 (0.80–1.47)
Luo et al (2017)[25]	215	298	244	303	11.5	0.63 (0.43–0.92)
Zhu et al (2017)[26]	349	505	328	510	15.5	1.24 (0.96–1.61)
Zhu (2016)[24]	255	396	249	378	14.3	0.94 (0.70–1.26)
Total (95% CI)		**3,082**		**3,044**	**100**	**0.93 (0.78–1.12)**
Total events	2,115		2,117			

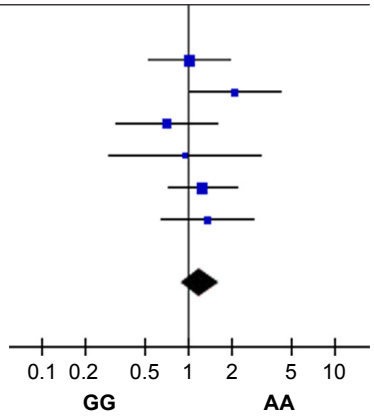
Heterogeneity: τ^2=0.04; χ^2=16.43, *df*=6 (*P*=0.01); I^2=63%
Test for overall effect: *Z*=0.77 (*P*=0.44)



D Homozygous model (GG vs AA)

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, fixed, 95% CI
Huang et al (2015)[20]	0	398	0	403		Not estimable
Jeon et al (2013)[16]	23	483	18	383	22.6	1.01 (0.54–1.91)
Liu et al (2014)[18]	19	200	14	292	12.2	2.08 (1.02–4.26)
Lyu et al (2016)[23]	11	268	15	265	17.1	0.71 (0.32–1.58)
Luo et al (2017)[25]	5	220	6	250	6.5	0.95 (0.28–3.14)
Zhu et al (2017)[26]	32	381	24	352	27.0	1.25 (0.72–2.17)
Zhu (2016)[24]	18	273	13	262	14.6	1.35 (0.65–2.82)
Total (95% CI)		**2,223**		**2,207**	**100**	**1.20 (0.90–1.60)**
Total events	108		90			

Heterogeneity: χ^2=4.47, *df*=5 (*P*=0.48); I^2=0%
Test for overall effect: *Z*=1.25 (*P*=0.21)



E Heterozygous model (AG vs AA)

Study or subgroup	Events	Total	Events	Total	Weight (%)	OR M–H, random, 95% CI
Huang et al (2015)[20]	133	531	128	531	15.2	1.05 (0.80–1.39)
Jeon et al (2013)[16]	195	655	170	535	16.4	0.91 (0.71–1.17)
Liu et al (2014)[18]	96	277	99	377	13.3	1.49 (1.06–2.09)
Lyu et al (2016)[23]	110	367	113	363	14.0	0.95 (0.69–1.30)
Luo et al (2017)[25]	78	293	53	297	11.6	1.67 (1.13–2.48)
Zhu et al (2017)[26]	124	473	158	486	15.2	0.74 (0.56–0.98)
Zhu (2016)[24]	123	378	116	365	14.3	1.04 (0.76–1.41)
Total (95% CI)		**2,974**		**2,954**	**100**	**1.06 (0.87–1.28)**
Total events	859		837			

Heterogeneity: τ^2=0.04; χ^2=17.10, *df*=6 (*P*=0.009); I^2=65%
Test for overall effect: *Z*=0.56 (*P*=0.57)

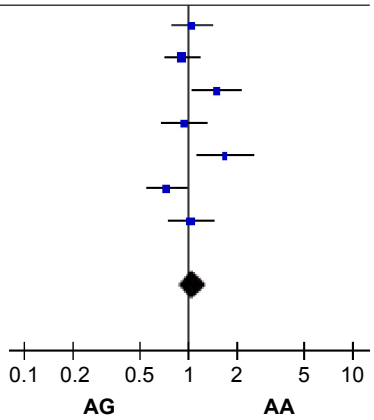


Figure 5 Forest plot describing the association between the miR-149 (rs2292832) polymorphism and ischemic stroke risk according to different genetic models: (**A**) allelic, (**B**) recessive, (**C**) dominant, (**D**) homozygous, and (**E**) heterozygous.

between the C allele of miR-146a (rs2910164) and lower IS risk contained only one case–control study, which was by Jeon et al.[16]

While the meta-analysis by Zhu et al[39] reported an association between the A allele of miR-499 (rs3746444) and decreased IS risk in Chinese, our meta-analysis did not detect this association, either across Asian populations or specifically in the Chinese population (data not shown). Our result may be more reliable because our meta-analysis included four more case–control studies[23–26] than the one by Zhu et al.[39] The results of our meta-analysis are consistent with those reported in the meta-analysis by Xiao et al.[37]

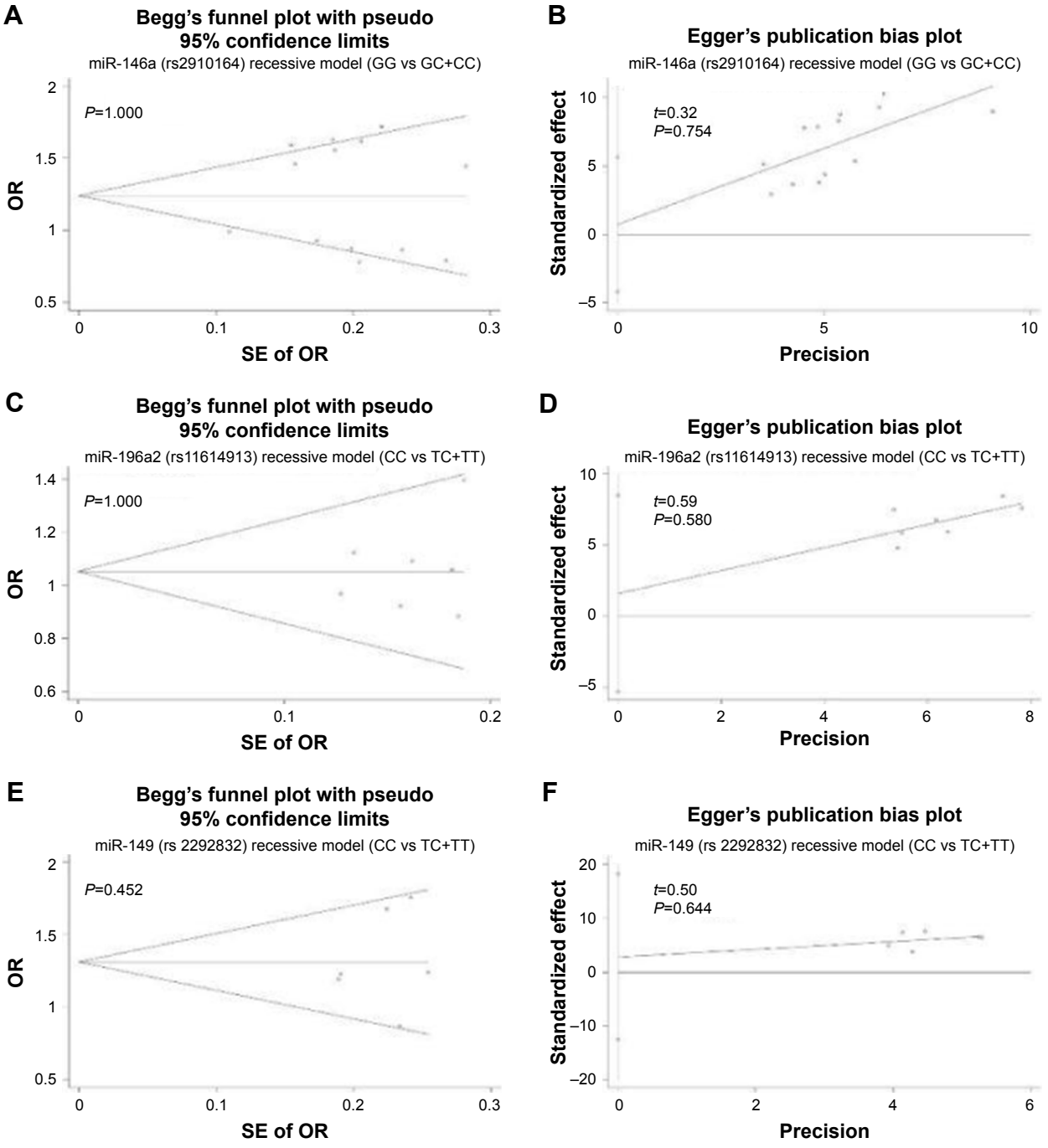


Figure 6 (*Continued*)

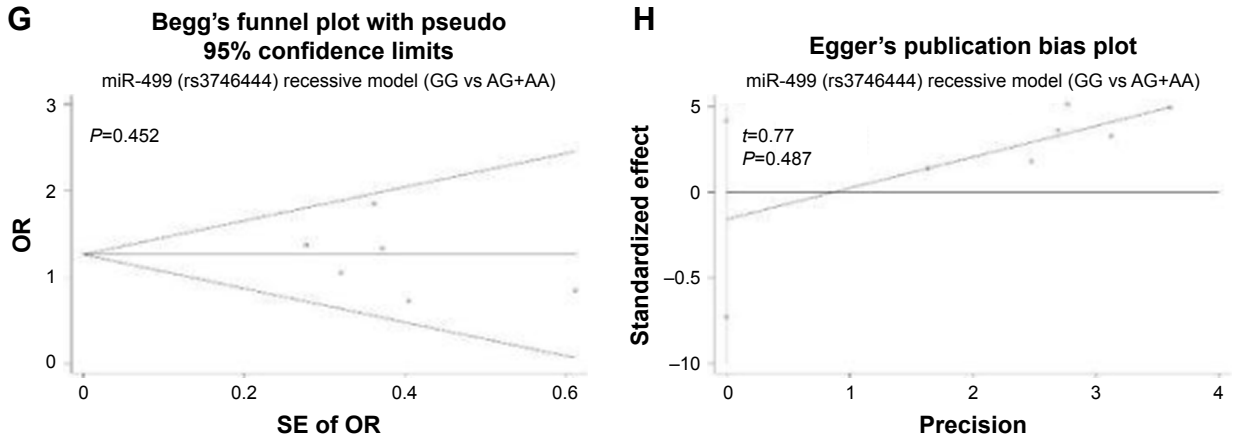


Figure 6 Begg's funnel plot and Egger's test to assess publication bias in the meta-analysis of potential associations between ischemic stroke risk and (**A** and **B**) miR-146a (rs2910164), (**C** and **D**) miR-196a2 (rs11614913), (**E** and **F**) miR-149 (rs2292832), and (**G** and **H**) miR-499 (rs3746444).
Note: All analyses were performed using a recessive genetic model.

Our meta-analysis suggests a significant association between the CC genotype of miR-149 (rs2292832) and increased IS risk. In contrast, the meta-analysis of Xiao et al[37] based on two case–control studies indicated that the TT genotype and T allele of miR-149 (rs2292832) are associated with significantly lower IS risk, whereas another meta-analysis[36] based on three case–control studies found the CC genotype and C allele of miR-149 (rs2292832) to be significantly associated with IS risk. Our meta-analysis contained three more case–control studies[24–26] than either of these other meta-analyses, which may make it more reliable.

Our meta-analysis did not find a significant association between miR-196a2 (rs11614913) polymorphism and IS risk. This result confirms other meta-analyses[37–39] based on smaller samples.

To the best of our knowledge, the current meta-analysis involves the largest sample (6,083 cases and 7,248 controls) than previous studies[36–39] investigating the possible association of IS risk with miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444) in Asian populations. Nevertheless, the meta-analysis is limited by the designs of the included studies. First, the *P*-value for HWE was less than 0.05 in two studies[14,22] on miR-146a (rs2910164) and one study[26] on miR-499 (rs3746444). These results suggested that these study populations may not be representative of the broader target population. Second, the results may be affected by both genetic and environmental factors, but most studies did not report environmental exposure, making it impossible to include them in the meta-analysis. Third, our exclusion of unpublished data and of papers published in languages other than English and Chinese may have biased our results. Fourth, the studies may be subject to performance bias, attrition bias, and reporting bias, although Newcastle–Ottawa scores were at least 5 for all 14 studies, indicating high quality. Fifth, stroke is a heterogeneous disease and has different subtypes that may affect the results of genetic association studies, but most case–control studies in our meta-analysis appeared not to use a well-phenotyped population. This may make the results less accurate. Finally, all the patients in this meta-analysis were Asian and this may limit the relevance of the results to other populations. Thus, more large and well-designed studies are warranted in non-Asian populations.

Conclusion

This meta-analysis suggests that the GG genotype of miR-146a (rs2910164) and the CC genotype of miR-149 (rs2292832) may confer increased susceptibility to IS in Asian populations, whereas polymorphism in miR-196a2 (rs11614913) and miR-499 (rs3746444) may not be associated with IS risk. These conclusions should be verified in large and well-designed studies.

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Author contributions

The study was designed by JRW and LC. The research was performed by DHZ, CBL, and QZ. Statistical analyses were

performed by XFL, GQ, QH, and YSM. The manuscript was written by DHZ. All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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