

Association between lncRNA ANRIL genetic variants with the susceptibility to ischemic stroke

From a case-control study to meta-analysis

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Abstract

Background: Recent studies have reported that lncRNA (long noncoding RNAs) antisense non-coding RNA in the INK4 locus (ANRIL) plays important roles in the development of atherosclerosis through regulating cell apoptosis, proliferation, and adhesion. GWAS (genome-wide association studies) identified common genetic variants within ANRIL could confer risk of ischemic stroke (IS) in southern Sweden.

Methods: We performed a case-control study, including 567 IS patients and 552 healthy controls from unrelated northern Chinese Han population, aiming to explore the association between lncRNA ANRIL rs2383207, rs4977574 polymorphisms and the risk of IS. Subsequently we implemented a meta-analysis to further assess the relationship of these variants and the disease.

Results: In our case-control study, no significant associations were observed in all models between above 2 polymorphisms and IS. Next in our subgroup analysis, we detected significant association between GA genotype of rs4977574 and the increased risk of LAA-IS (large-artery atherosclerotic ischemic stroke), similar elevated risk also appeared in the GG+GA genotype under the dominant model ($P=.048$, OR=1.385, 95% CIs 1.002–1.914; $P=.040$, OR=1.378, 95% CIs 1.015–1.872, respectively). As for rs2383207, negative results were obtained under all models and subgroups. Our meta-analysis showed a significant association between rs4977574 polymorphism and IS risk in allele model (G vs A $P=.002$, OR=1.137, 95% CIs 1.048–1.234); with respect to rs2383207 polymorphism, no significant association between that and the risk of IS was detected under the dominant model (GA + AA vs GG, $P=.061$, OR=0.923, 95% CIs 0.849–1.004), or recessive model (AA vs GA + GG, $P=.656$, OR=0.972, 95% CIs 0.858–1.101), or allele model (A vs G, $P=.326$, OR=0.952, 95% CIs 0.863–1.050). Likewise, no significant association between rs2383207 and IS was found in different stroke subtypes ($P>.05$).

Conclusions: Our findings indicated G allele of lncRNA ANRIL rs4977574 could increase the risk of IS, and the variant may be associated with susceptibility to LAA-IS in Chinese Han population.

Abbreviations: 95% CIs = 95% confidence intervals, ANRIL = antisense non-coding RNA in the INK4 locus, BMI = body mass index, CAD = coronary artery disease, GWAS = genome-wide association studies, IS = ischemic stroke, LAA-IS = large-artery atherosclerotic ischemic stroke, lncRNA = long noncoding RNAs, ORs = odds ratios, SAO-IS = small-artery occlusive ischemic stroke.

Keywords: case-control study, ischemic stroke, long noncoding RNAs, antisense non-coding RNA in the INK4 locus, meta-analysis, rs2383207, rs4977574

1. Introduction

Stroke is a life-threatening issue with high morbidity and mortality, and has become a public burden especially for elder

people in the past decades. Among the various types, ischemic stroke (IS) contributes to 87% roughly, which is a multi-factorial disease influenced by both genetic and environmental factors

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containing difficult pathophysiological mechanisms.^[1–3] It has been proved that many pathophysiological courses closely relating to cardiovascular disease in the previous studies are also involved into the progression of IS, such as atherosclerosis, immunological and inflammatory responses, apoptosis.^[4–7] The restoration of blood supply to ischemic penumbral tissue is an essential treatment target. Intravenous thrombolysis and mechanical thrombectomy are main therapeutic methods in the acute phase with some limitation including narrow treatment window and reperfusion injury.^[8] Thus, it is urgent to advance the development of new therapies. Recently, researches have declared that long noncoding RNAs (lncRNAs) are involved in various courses during the progression of IS.

lncRNAs are defined as non-protein-coding transcripts of more than 200 nucleotides, and have been attracted increasing attention because of their cell and tissue specificity.^[9] lncRNAs comprise a vast amount of genomes and play valuable roles in normal physiology and disease, mainly by transcriptional regulation in cis or trans, organization of nuclear domains, and regulation of proteins or RNA molecules.^[10] lncRNAs also affect miRNA functions by means of controlling premRNA splicing or as miRNA sponges. Mounting researches have revealed lncRNAs would significantly differentially expressed in IS patients with the advance of disease course.^[11] Besides that, lncRNAs are associated with several processes such as atherosclerosis, dyslipidemia, hypertension, and diabetes mellitus, leading to the occurrence of IS ultimately.^[12]

lncRNA ANRIL (antisense non-coding RNA in the INK4 locus, CDKN2BAS) is an important part located at chromosome 9p21 (Chr9p21), a locus recognized in the first surge of (GWAS) genome-wide association studies of coronary artery disease (CAD), the changes of whose abundance were put forward that could have relevance to atherosclerosis risk through regulating apoptosis, cell proliferation and cell adhesion. ANRIL, known as co-clustered with cyclin-dependent kinase inhibitor (CDKN2A and CDKN2B) and alternate reading frame, was found to activate the NF- κ B signaling pathway and promote expression of proinflammatory cytokines, which are vital developments in IS progression.^[13] In Feng study, they discovered that the level of plasma lncRNA ANRIL correlated with risk and severity of stroke.^[14] GWAS identified common genetic variants, ANRIL polymorphism rs2383207 could confer risk of IS in southern Sweden, another polymorphism rs4977574 might be responsible for the development of CAD in Turkish Cypriot population and Asian population. What is more, a study consisting of GWAS case-control samples by Dichgans et al demonstrated that there were existing in shared genetic influences between IS and CAD, and 9p21.3 genetic locus were pointed out in it.^[15–18] Therefore, we selected 2 variants rs2383207, rs4977574 and did a case-control study to explore the relationship between those and the susceptibility to IS in Chinese Han population. Whereafter, we conducted a meta-analysis from all eligible case-control studies to further assess the conclusion and provide a novel window of opportunity to guide clinical practice in IS therapeutic.

2. Materials and method

2.1. Case-control study

2.1.1. Subjects. Five hundred sixty seven IS patients and 552 healthy controls were incorporated into our case-control study, in which, patients were hospitalized in Department of Neurology,

the First Affiliated Hospital of China Medical University, while controls were collected in the Red Cross hospital who have no history of neurological system-related diseases, all above subjects were unrelated Chinese Han population in the Liaoning Province of northern China. Eligible cases were patients who diagnosed with acute ischemic stroke for the first time as the first diagnosis with methods of clinical manifestation, neurological examination, and radiological imaging (computed tomography and magnetic resonance imaging). Patients with transient ischemic attack, cerebral embolism, cerebral trauma, cerebrovascular malformations, coagulation disorders, autoimmune diseases, tumors, chronic infection diseases, chronic metabolic diseases, and peripheral vascular disease were all ruled out. Our cases were divided into 2 subtypes, those were large-artery atherosclerotic ischemic stroke (LAA-IS) and SAO-IS (small-artery occlusive ischemic stroke), cardioembolism, stroke of other determined or undetermined causes were all excluded, because genetic factors appear to be more important in large-artery and small-vessel stroke than in other subtypes.^[19] Furthermore, this study was approved by the ethics committee of the First Affiliated Hospital of China Medical University approval, in accordance with the principles of the Helsinki Declaration (AF-SOP-07-1.0-01). Written informed consents were obtained from all the participants.

2.1.2. Genotyping. A whole-blood DNA extraction kit was applied to extract the genomic DNA of peripheral blood sample according to the manufacturer's instructions. The rs2383207 and rs4977574 polymorphism was determined by polymerase chain reaction-ligation detection reaction (PCR-LDR) method, which was same as our published manuscript.^[20]

2.2. Statistical analysis

All genotype frequencies were further examined by Hardy-Weinberg analysis in cases and controls through χ^2 test. Odds ratios (ORs) and corresponding to 95% confidence intervals (95% CIs) were used to evaluate the association between ANRIL polymorphisms rs2383207, rs4977574 and the risk of IS. Multivariate logistic regression analyses were utilized to exclude from confounding factors to the influence of polymorphisms initiating consequences. All the statistical analyses were carried out by means of SPSS version 23.0, within that, the *P* value of .05 was considered statistically significant.

2.3. Meta-analysis

2.3.1. Search strategy and Inclusion criteria. In our meta-analysis, 8 researches for rs2383207 and 8 researches for rs4977574 were included until September 2020 from MEDLINE, EMBASE, and PubMed after selecting and excluding strictly. Potentially relevant articles, whose deadline was 30 September 2020, were retrieved from the electronic databases using the following terms: “stroke” and “rs2383207 or rs4977574”. References were also checked for other potentially relevant literatures which were not found in the original search. Inclusion criteria were described as following clause:

1. studies aimed at the association between rs4977574, rs2383207 and stroke using an unrelated case-control design;
2. genotypic or allelic frequency or number of rs4977574, rs2383207 could be obtained in cases and controls;
3. full-text studies written in English;

4. studies in which there was agree with the Hardy–Weinberg equilibrium among the controls.

2.3.2. Data extraction. The following information was extracted from included literatures: name of first author, year of publication, country and ethnicity of study, clinical characteristics and sample size of cases and controls, genotyping and corresponding numbers of each SNP in cases, and controls. The probability value (P value) of Hardy–Weinberg equilibrium was listed. Data extraction from each study and data input were fulfilled by 2 authors independently. Disagreements were resolved by consensus.

2.4. Statistical analysis

Study heterogeneity was assessed by I^2 statistics, of which $I^2 < 50\%$ meant to present fixed-effects model, otherwise random-effects model were used. Odds ratios and 95% CIs were used to evaluate the strength of associations. In addition, we constructed 3 models (dominant, recessive, allele) and 2 subgroups (LAA-IS, SAO-IS) to describe genotype and allele frequencies, within that significance set at $P < .05$. Publication bias was detected by Begg funnel plot and Egger test. STATA 15.0 were used to analyze data.

3. Results

3.1. Results of case-control study

In our study, 567 IS patients and 552 healthy controls met the criteria for inclusion, who were all from unrelated northern Chinese Han population. In Table 1, there were summarized that clinical characteristics of the participants according to the case and control groups. Over all, age, gender, or body mass index (BMI) showed no significant influence of IS ($P > .05$). In reference to hypertension, diabetes, hypercholesterolemia, smoking, and alcohol drinking, which were significantly higher, as compared with controls, demonstrating that they could be considered as risk factors for the disease ($P < .05$).

The genotype and allele frequencies of 2 lncRNA ANRIL gene polymorphisms in the case and control groups are presented in Tables 2 and 3, moreover, genotype and allele distribution of those in the subtype analysis of IS were shown in Tables 4 and 5. The distribution of the ANRIL genotype did not deviate from the Hardy–Weinberg equilibrium ($P > .05$). For rs2383207, P value was .750 and .949 in the case and control groups, respectively; as for rs4977574, P value was .155 and .584 in the case and control groups, respectively.

Table 1

Clinical characteristics of the participants.

	Case (n = 567)	Control (n = 552)	P value
Age(mean ± SD)	61.72 ± 10.17	61.9 ± 9.52	.677
Male gender, n (%)	364 (64.2%)	348 (63.0%)	.668
Hypertension, n (%)	396 (69.8%)	320 (57.9%)	.000
Diabetes, n (%)	213 (37.5%)	124 (22.3%)	.000
Hypercholesterolemia, n (%)	259 (45.7%)	204 (36.9%)	.003
Smoking, n (%)	204 (35.9%)	107 (19.4%)	.000
Alcohol drinker (%)	113 (19.9%)	65 (11.8%)	.000
BMI (kg/m ²)	25.05 ± 3.70	25.34 ± 3.30	.285

BMI = body mass index, SD = standard deviation.

Table 2

Genotype and allele distributions of ANRIL rs2383207 for the patients with IS and the control group (G > A).

	Case	Control	P	OR	95%CI
Genotype					
GG	253	243	Reference		
GA	254	247	.922	0.988	0.771–1.266
AA	60	62	.718	0.929	0.625–1.381
Dominant effect					
GA+AA vs GG	253/314	243/309	.840	0.976	0.771–1.236
Recessive effect					
AA vs GA+GG	60/507	62/490	.727	0.935	0.642–1.362
Allele					
G	760	733	Reference	0.972	
A	374	371	.754		0.815–1.159

In Table 2, we did not discover significant association between the GA, AA genotypes of rs2382207 (G > A) with IS (OR = 0.988, 95%CI = 0.771–1.266, $P > .05$; OR = 0.929, 95%CI = 0.625–1.381, $P > .05$). What's more, no significant association between the rs2383207 and the risk of IS was detected under the dominant model (GA+AA vs GG, $P = .840$, OR = 0.976, 95% CIs 0.771–1.236), or recessive model (AA vs GA+GG, $P = .727$, OR = 0.935, 95% CIs 0.642–1.362), or allele model (A vs G, $P = .754$, OR = 0.972, 95% CIs 0.815–1.159). As regards variant 4977574 (A > G), data in Table 3 displayed that the GA and GG genotypes did not contribute to the susceptibility to IS (OR = 1.267, 95%CI = 0.959–1.673, $P > .05$; OR = 1.151, 95%CI = 0.822–1.611 $P > .05$), similar results were obtained in dominant model, recessive model, and allele model (GA+GG vs AA, $P = .122$, OR = 1.231, 95% CIs 0.946–1.601; GG vs GA+AA, $P = .927$, OR = 0.987, 95% CIs 0.743–1.311; G vs A, $P = .355$, OR = 1.082, 95% CIs 0.916–1.277).

Subsequently, we did a subtype analysis to categorize IS into LAA-IS and SAO-IS according to TOAST classification, in order to determine whether these polymorphisms provided a link between the susceptibility to specific stroke subtype. As shown in Table 4, we did not catch sight of any significant differences between the rs2383207 and LAA or SVD subgroups under the allelic and genotypic models ($P > .05$). For rs4977574, the GA genotype of rs4977574 showed noticeable association with the risk of LAA-IS ($P = 0.048$, OR = 1.385, 95%CI = 1.002–1.914), which is an indication that individuals with GA genotype had a

Table 3

Genotype and allele distributions of ANRIL rs4977574 for the patients with IS and the control group (A > G).

	Case	Control	P	OR	95%CI
Genotype					
AA	144	163	Reference		
GA	300	268	.095	1.267	0.959–1.673
GG	123	121	.414	1.151	0.822–1.611
Dominant effect					
GG+GA vs AA	144/423	163/389	.122	1.231	0.946–1.601
Recessive effect					
GG vs GA+AA	123/444	121/431	.927	0.987	0.743–1.311
Allele					
A	588	594	Reference	1.082	
G	546	510	.355		0.916–1.277

Table 4**Genotype and allele distributions of LncRNA ANRIL polymorphism rs2383207 in the ischemic stroke subtypes.**

	LAA					SVO				
	Case	Control	P	OR	95%CI	Case	Control	P	OR	95%CI
Genotype										
GG	159	243	Reference			94	243	Reference		
GA	159	247	.910	0.984	0.742–1.305	95	247	.973	0.994	0.711–1.391
AA	38	62	.776	0.937	0.597–1.470	22	62	.755	0.917	0.534–1.576
Dominant effect										
GA + AA vs GG	159/197	243/309	.849	0.974	0.745–1.274	94/117	243/309	.895	0.979	0.711–1.347
Recessive effect										
AA vs GA + GG	38/318	62/490	.793	0.944	0.616–1.449	22/189	62/490	.751	0.920	0.550–1.539
Allele										
G	477	733	Reference	0.973	0.797–1.189	283	733	Reference	0.970	
A	235	371	.791			139	371	.805		0.765–1.232

Table 5**Genotype and allele distributions of LncRNA ANRIL polymorphism rs4977574 in the ischemic stroke subtypes.**

	LAA					SVO				
	Case	Control	P	OR	95%CI	Case	Control	P	OR	95%CI
Genotype										
AA	83	163	Reference			61	163	Reference		
GA	189	268	.048	1.385	1.002–1.914	111	268	.589	1.107	0.766–1.599
GG	84	121	.114	1.363	0.929–2.001	39	121	.529	0.861	0.541–1.372
Dominant effect										
GG + GA vs AA	83/273	163/389	.040	1.378	1.015–1.872	61/150	163/389	.867	1.030	0.727–1.461
Recessive effect										
GG vs GA + AA	84/272	121/431	.556	1.100	0.801–1.510	39/172	121/431	.297	1.238	0.828–1.851
Allele										
A	355	594	Reference	1.171	0.970–1.414	233	594	Reference	0.945	
G	357	510	.100			189	510	.621		0.754–1.184

1.385-fold incremental risk than those with AA genotype in the LAA IS. However, individuals with GG genotype did not show increased risk with reference to those were AA genotype in the LAA-IS subgroup. Similar positive results were also observed in the dominant effect, with genotype AA as a referential standard, GG + GA genotype contributed to a 1.378-fold increased risk in the large-artery atherosclerotic stroke ($P=.040$, $OR=1.378$, $95\%CI=1.015-1.872$). Nevertheless, as for ANRIL polymorphism rs4977574, no evident statistically differences between cases and controls under the recessive and allelic models of LAA-IS. Similarly, in the SVD subtype, we did not detect significant association of rs4977574 with the incidence of IS under any genetic models ($P>.05$). (Table 5)

In order to exclude the influence of hypertension, diabetes, or other potential confounders for rs4977574 in LAA-IS under the dominant model, we did a multivariate logistic regression analysis, results were showed in Table 6. Noteworthy association

between GA + GG genotype with the increased frequency of LAA-IS was still taken on after precluding confounding factors (age, male gender, hypertension, diabetes, hypercholesterolemia, smoking, alcohol drinking, BMI) ($P=.041$, $OR=1.395$, $95\%CI=1.013-1.921$).

3.2. Results of meta-analysis

For rs2383207, a total of 8 literatures were included, we selected 6 of them to analysis the association between the variant and the risk of IS, 5 of them to analysis the relationship between that and LAA-IS, while 3 of them aiming at SAO-IS. Eight researches were enrolled to investigate potential roles of rs4977574 in IS. What is more, the characteristics of included articles were displayed in Table S.1 (supplemental materials, <http://links.lww.com/MD/F893>).

With respect to rs2383207, I^2 were 20.9%, 47.7%, and 55.3% in the study heterogeneity under the dominant, recessive, and allele models, respectively. Moreover, no significant association between rs2383207 and the risk of IS was detected under the dominant model (GA + AA vs GG, $P=.061$, $OR=0.923$, $95\%CI$ s 0.849–1.004), or recessive model (AA vs GA + GG, $P=.656$, $OR=0.972$, $95\%CI$ s 0.858–1.101), or allele model (A vs G, $P=.326$, $OR=0.952$, $95\%CI$ s 0.863–1.050) (Figs. 1–3, shown in supplemental materials, <http://links.lww.com/MD/F888>, <http://links.lww.com/MD/F889>, <http://links.lww.com/MD/F890>). When we conducted a further analysis under different stroke subtypes, no significant association were observed in all genetic models (Figs. 4–5, shown

Table 6**Ischemic stroke risk factors in the logistic regression analysis.**

	P	OR	95%CI
Hypertension	.058	1.337	0.990–1.806
Diabetes	.000	1.932	1.426–2.617
Smoking	.000	2.258	1.603–3.180
Genotype GG + GA of ANRIL rs4977574	.041	1.395	1.013–1.921

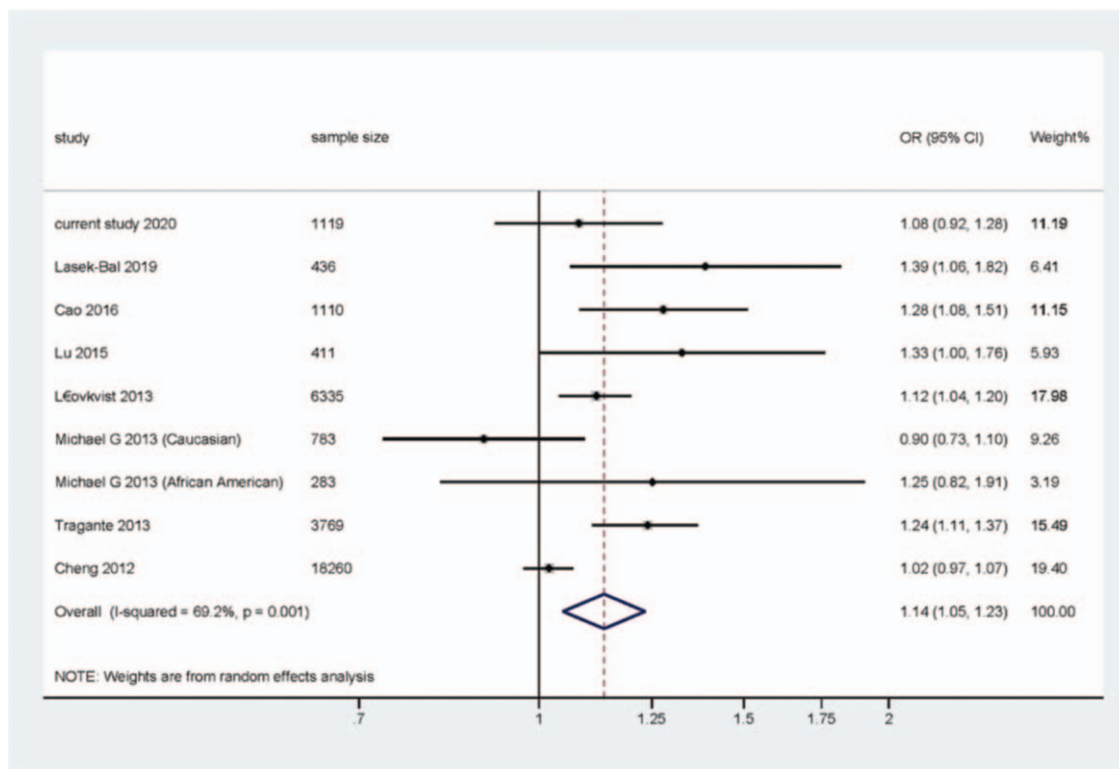


Figure 1. Random-effects meta-analysis for the association between the rs4977574 polymorphism and the susceptibility to IS under the allele model (G vs A).

in supplemental materials, <http://links.lww.com/MD/F891>, <http://links.lww.com/MD/F892>). However, significant association between rs4977574 and the risk of IS could be recognized in allele model (G vs A, $P=.002$, OR=1.137, 95% CIs 1.048–1.234) (Fig. 1). These results indicated that the G allele frequency was higher in the ischemic patients than that in controls, and the risk for

individuals with G allele was 1.137-fold higher than those with A allele. I^2 was 69.2% in the study heterogeneity under the allele model for rs4977574. Then we constructed a Galbraith graph to explore relatively high heterogeneity and found study Cheng 2012 and Tragante 2013 contributed to a large proportion of heterogeneity (Fig. 2).

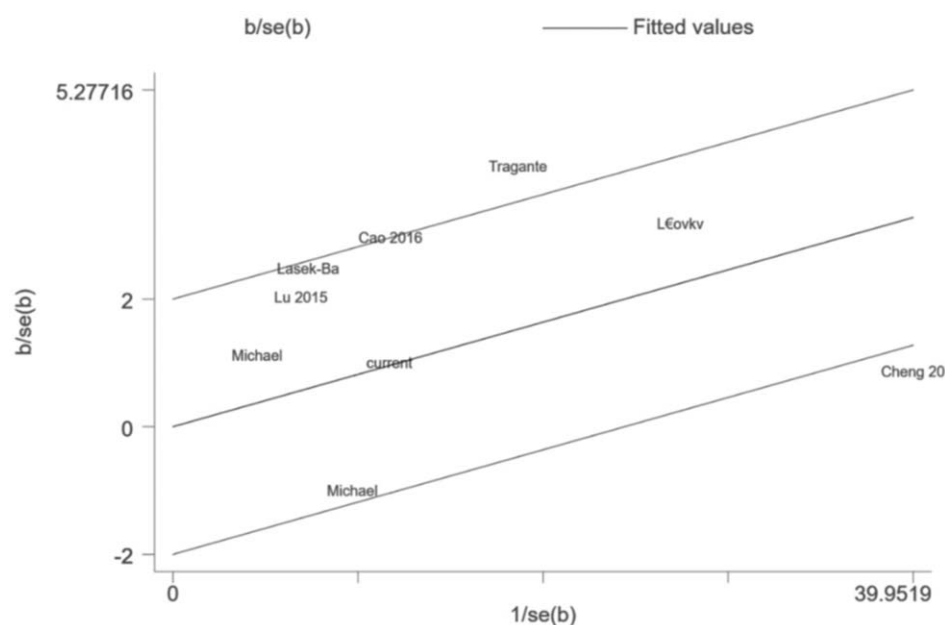


Figure 2. Galbraith graph of the association between the rs4977574 polymorphism and the susceptibility to IS under the allele model (G VS A).

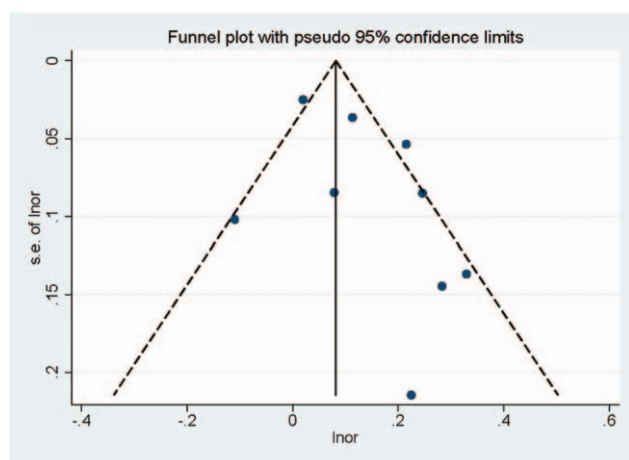


Figure 3. Forest plot of the association between the rs4977574 polymorphism and the susceptibility to IS under the allele model (G VS A).

3.3. Publication biases

Begg funnel plot and Egger test were applied to assay publication bias, while significance set at $P < .1$. As regard to rs2383207, P value of Begg test and Egger test is shown in Table S.2 (supplemental materials, <http://links.lww.com/MD/F894>), no obvious publication bias was discovered ($P > .1$). The shape of the abovementioned funnel plots is seemed symmetrical (not shown). The results of Begg funnel plot and Egger test for rs4977574 were 0.917 and 0.139 under the allele model (Fig. 3), separately.

3.4. Sensitivity analysis

We performed sensitivity analysis to test the pooled results of our meta-analysis. As shown in Figure 4, the corresponding pooled

OR did not altered significantly when we removed each study at a time, indicating that our meta-analysis obtained a relatively stable and credible result (Fig. 5).

4. Discussion

In our case-control study, we did a genotypic analysis among 567 individuals which were diagnosed as IS and 552 healthy controls in Chinese Han population, and showed that no significant association between SNP rs2383207, rs4977574 and the susceptibility to IS under dominant, recessive, and allele models. Later in our subgroup analysis, it was worth noting that we discovered the GA genotype of rs4977574 showed significant association with the susceptibility to LAA-IS, besides that, similar positive results appeared in GG+GA genotype under the dominant model. While in other models, we did not identify conspicuous relevance between genetic variant rs4977574 and IS. For polymorphism rs2383207, we did not detect positive conclusions neither in the subtype LAA-IS nor SAO-IS.

Recent studies showed that polymorphisms have played fascinating roles in the process of IS.^[21,22] As for the connection between SNP rs2383207, rs4977574 and the risk of IS, there are existing positive conclusions in some researches, negative results in others.^[23–32] Meta-analysis is an effective method that could enlarge sample size and reduce false-positive or false-negative results on account of random error. Thus, we have conducted this meta-analysis to derive a more convincing assessment of the association between above 2 SNP polymorphisms and the risk of IS. Our meta-analysis showed significant correlation between rs4977574 and the susceptibility to IS under the allele model. Furthermore, we found a higher allele frequency of G allele in IS patients compared with controls, the risk of individuals with that was 1.137-fold higher than participants with A allele. Our Egger test and sensitivity analysis suggest the stability and reliability of our included data and obtained conclusions. Therefore, we investigated that the G allele of rs4977574 could

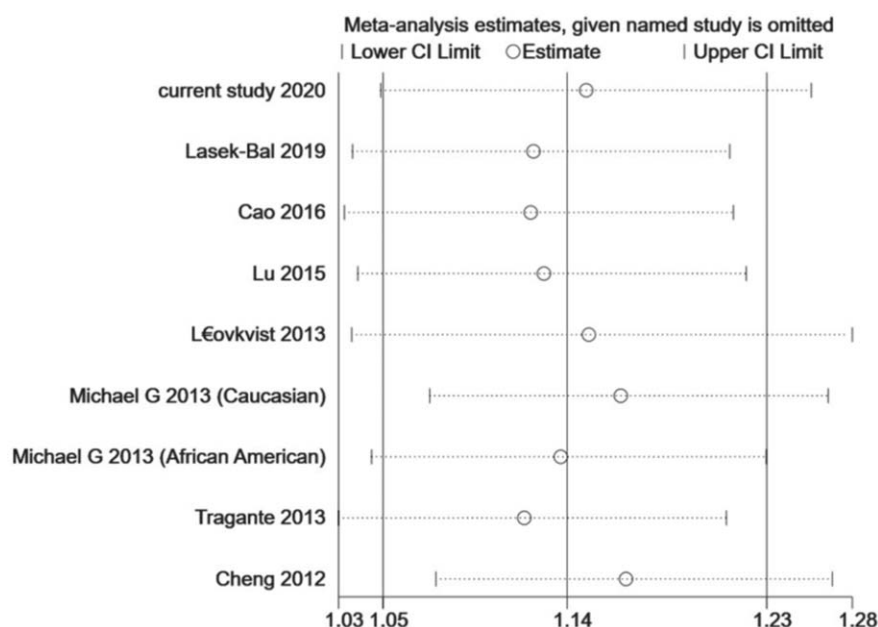


Figure 4. The influence of each research by removal of individual researches for rs4977574 under the allele model (G VS A).



PRISMA 2009 Flow Diagram

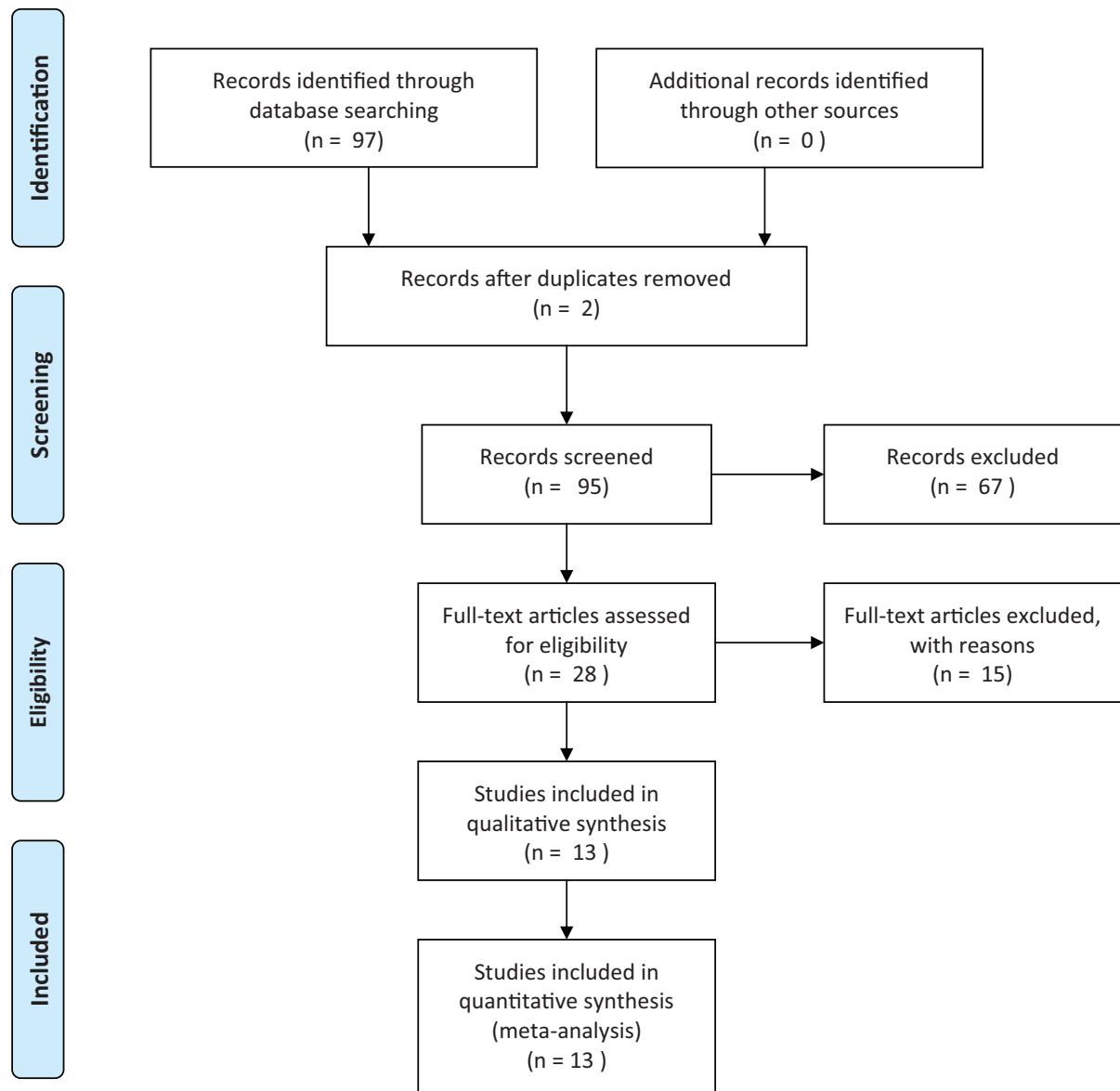


Figure 5. The flow diagram of our meta-analysis.

increase the risk of IS. A previous multiethnic study of atherosclerosis revealed that significant associations for coronary artery calcium with rs4977574 in Caucasians and Hispanic, however, positive results were not found in Chinese and African Americans. Their findings suggested there existing some shared genetic architecture, as well as novel variants and pathways with the vicissitude of environment and time among diverse ethnic population.^[33] In our meta-analysis, the heterogeneity of rs4977574 was relatively high ($I^2=69.2\%$), the reasons for that might be afore-mentioned racial factors and

environmental influence, furthermore, differences in sample sizes of included literatures might contribute to a part of it. Our Galbraith graph showed study Cheng 2012 and Tragante 2013 contributed to a large proportion of heterogeneity. When we removed of these 2 studies, the I^2 in the study heterogeneity descended to 47.1% and positive result was still obtained. With respect to rs2383207, there is no significant association was detected in all models and subgroups. To our knowledge, this is the initial meta-analysis which explores the connection between LncRNA ANRIL variant rs4977574 and IS, further brings to

light that G allele within rs4977574 performing an increased risk in the occurrence and development of IS.

In our meta-analysis, allele G of rs4977474 was recognized as risk allele increasing the susceptibility to IS, GA genotype and GG + GA genotype under the dominant model of rs4977574 showed significant association with the LAA-IS but not SAO-IS in our case-control study. LAA-IS and SAO-IS are represented by different pathophysiologic mechanisms, the former is attributable to the formation of atherosclerotic plaque in the carotid large artery or skull base moderately large artery, the latter is mostly due to hypertension-causing small perforating artery hyalinization or vascular sclerosis.^[20] There might be existing several potential mechanisms in the involvement and regulation of rs4977574 contributing to the pathophysiology of IS. In a recent research, the risk allele of rs4977574 was found to be related to higher level of serum total cholesterol and lower level of high-density lipoprotein-cholesterol in the CAD group of a Turkish Cypriot population. It is well known that Low HDL-C level is strongly coincided with the development of intracranial atherosclerotic stenosis. These findings suggested that the risk allele rs4977574 might contribute to occurrence of atherosclerosis.^[16,34] Recent research declared that risk allele of rs4977574 probably associated with carotid plaque formation in acute IS patients.^[35] Moreover, another article found that G allele of rs4977574 was linked with higher levels of fasting serum insulin in young women from different ethnocultural groups, which would increase the susceptibility to atherosclerosis and subsequent type 2 diabetes, further might contribute to the occurrence of LAA-IS.^[36] In addition, Kunnas et al elucidated that GG genotype within rs4977574 was correlated with the decreased risk of hypertension aiming at Tampere adult population, these findings and other pathogenic factors in combination might plausibly relate to our conclusions that no significant increased risk were detected in the SVD-IS subtype among individuals with GG genotype of rs4977574.^[37] Above all, we made assumptions about the risk allele G would play important roles mainly in dyslipidemia, carotid plaque formation, type 2 diabetes, and lead to the progression of atherosclerotic vascular diseases and IS.

Allele A of rs2383207 was reported that could be related with the increased risk of LAA-IS in a Brazilian population,^[38] while similar results were not concluded in our case-control study aiming at Chinese Han population. In 2018, a meta-analysis revealed that no significant association between rs2383207 and IS in overall population, most of them are Asians and Caucasians (dominant comparison, $P=.64$, OR=1.02, 95%CI 0.93–1.13; recessive comparison, $P=.79$, OR=0.98, 95%CI 0.85–1.13; allele comparison, $P=.21$, OR=1.04, 95%CI 0.98–1.10).^[39] In our meta-analysis, we complemented some more articles for genotypic and allelic analysis and came to a similar conclusion. We did a subgroup analysis to explore the relationship between the variant and specific stroke subtypes, however, negative results were presented in the LAA-IS and SAO-IS subgroups under all genetic models. This is the first meta-analysis to assess the association between rs2383207 and the risk of specific stroke subtypes, we did not obtain positive results according to current studies, further investigations are needed in the future.

Some limitations of this meta-analysis should be listed. First, detailed analysis of the association between rs4977574 and IS susceptibility under a dominant model and a recessive model were not established on account of lacking of specific data. Likewise, we did not present a subgroup analysis of IS to explore the correlation between specific subtype of IS and rs4977574.

Second, we did not investigate the racial and environmental effects due to the limitation of data.

5. Conclusion

In our case-control study, we proposed that the GA genotype and GG + GA genotype under the dominant model of lncRNA ANRIL polymorphism rs4977574 presented an impellent role in progress of large artery atherosclerotic ischemic stroke in the Chinese Han population. Subsequently, in the meta-analysis we presented that G allele of rs4977574 could increase the risk of IS. Further analysis would be warranted to explore underlying mechanisms profoundly so that verify and advance our understanding in the association between ANRIL and IS.

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