Research Article

Effects of ANRIL variants on the risk of ischemic stroke: a meta-analysis

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Background: Several studies investigated the relationship between antisense non-coding RNA in the INK4 locus (ANRIL) variants and the risk of ischemic stroke (IS), yet whether ANRIL variants are associated with IS remain controversial. Therefore, we performed the present study to obtain a more conclusive result. Methods: Literature retrieval was conducted in PubMed, Medline and Embase. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Results: Eighteen studies were enrolled for analyses. Pooled overall analyses showed that rs2383206 (recessive model: P=0.002, OR = 1.22, 95%CI 1.08–1.38; allele model: P=0.003, OR = 0.90, 95%CI 0.84–0.96) and rs10757274 (allele model: P=0.006, OR = 0.91, 95%CI 0.86–0.97) variants were significantly associated with an increased risk of IS. Further subgroup analyses by ethnicity revealed that rs2383206, rs10757274 and rs10757278 variants were all significantly correlated with an increased risk of IS in Asians. Additionally, rs10757278 polymorphism was also significantly correlated with an increased risk of IS in Caucasians. Conclusions: Our findings indicated that rs2383206, rs10757274 and rs10757278 variants may impact individual susceptibility to IS in Asians. Moreover, rs10757278 polymorphism may also impact individual susceptibility to IS in Caucasians.

Introduction

Ischemic stroke (IS) is one of the leading causes of morbidity and mortality all over the world [1]. So far, the exact cause of IS remains ambiguous in spite of extensive investigations. Nevertheless, accumulating evidence suggests that genetic factors may play crucial parts in its pathogenesis. First, numerous genetic variants were found to be associated with an increased risk of IS by previous genetic association studies [2–4]. Second, screening of common causal variants was also proved to be a cost-efficient way to predict the individual risk of developing IS [5,6]. Overall, these findings supported that genetic predisposition is crucial for the occurrence and development of IS.

Antisense non-coding RNA in the INK4 locus (ANRIL) is located on human chromosome 9p21, a region that has been repeatedly linked to atherosclerosis and its associated ischemic vascular diseases [7]. Previous studies demonstrated that the expression levels of several neighbor protein-encoding genes like cyclin-dependent kinase inhibitors 2A (CDKN2A), CDKN2B and methylthioadenosine phosphorylase (MTAP) are modulated by ANRIL. It was shown that the above-mentioned proteins were abundantly expressed in atherosclerotic lesions, and they could promote atherosclerosis by impacting vascular remodeling, thrombogenesis and plaque stability [8]. Additional, recent experimental analyses also showed that ANRIL could promote inflammation by inhibiting caspase recruitment domain family member (CARD) 8 and activating the NF-κB pathway [9]. Considering the critical role of ANRIL in regulating atherosclerosis and inflammation as well as the close relationship between these two processes and IS, it is believed that functional ANRIL variants may also be involved in the development of IS.
In the past decade, several studies have already investigated potential correlations between ANRIL variants and the risk of IS, yet the results of these studies were controversial [10,11]. Thus, we performed the present meta-analysis to better evaluate the roles of ANRIL variants in IS.

**Materials and methods**

**Literature search and inclusion criteria**

This meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guideline [12]. The authors conducted a systematic search of PubMed, Medline and Embase to identify potentially related literatures published up to October 2018 using the following searching strategy: (antisense noncoding RNA in the INK4 locus OR CDKN2B antisense RNA OR ANRIL OR CDKN2B-AS long non-coding RNA) AND (polymorphism OR variant OR mutation OR genotype OR allele) AND (ischemic stroke OR cerebral infarction OR brain infarction OR cerebrovascular disease). Furthermore, the references of retrieved articles were also screened for other potentially relative studies.

To test the research hypothesis of this meta-analysis, included studies must meet all the following criteria: (a) case–control study on correlation between ANRIL variants and IS; (b) it provides genotypic and/or allelic frequency of ANRIL variants in cases and controls; (c) full text in English or Chinese available. Studies were excluded if one of the following criteria was fulfilled: (a) not related to ANRIL variants and IS; (b) case reports or case series; (c) abstracts, reviews, comments, letters and conference presentations. For duplicate reports, we only included the study with the largest sample size for analyses.

**Data extraction and quality assessment**

The following data were extracted from included studies: (i) name of the first author; (ii) publication year; (iii) country and ethnicity; (iv) sample size; and (v) genotypic distribution of ANRIL variants in cases and controls. Additionally, the probability value (P-value) of Hardy–Weinberg equilibrium (HWE) was also calculated. When necessary, we wrote to the corresponding authors for raw data. We used the Newcastle–Ottawa scale (NOS) to evaluate the quality of eligible studies [13]. This scale has a score range of zero to nine, and studies with a score of more than seven were thought to be of high quality. Two reviewers conducted data extraction and quality assessment independently. Any disagreement between two reviewers was solved by discussion until a consensus was reached.

**Statistical analyses**

All statistical analyses were achieved using Review Manager Version 5.3.3 (The Cochrane Collaboration, Software Update, Oxford, United Kingdom). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate strength of associations, and P-values ≤0.05 were considered to be statistically significant. Between-study heterogeneities were evaluated with I² statistic. Random-effect models (REMs) would be used to pool the data if I² ≥ 50%. Otherwise, fixed-effect models (FEMs) would be employed for synthetic analyses. Subgroup analyses by ethnicity were subsequently performed. Sensitivity analyses were executed to test the stability of synthetic results. Funnel plots were used to assess publication biases.

**Results**

**Characteristics of included studies**

We found 115 potential relative articles. Among these articles, a total of 18 eligible studies which met our inclusion criteria were included for synthetic analyses (see Figure 1). The NOS score of eligible articles ranged from 7 to 8, which indicated that all included studies were of high quality. Baseline characteristics of included studies were shown in Table 1 [10,14–28].

**Overall and subgroup analyses**

To investigate potential correlations between ANRIL variants and the risk of IS, six studies about rs1333040 polymorphism (2552 cases and 3359 controls), four studies about rs1333049 polymorphism (1543 cases and 2888 controls), five studies about rs2383206 polymorphism (2987 cases and 3600 controls), six studies about rs2383207 polymorphism (3793 cases and 8372 controls), seven studies about rs10757274 polymorphism (3106 cases and 16440 controls) and ten studies about rs10757278 polymorphism (6051 cases and 24199 controls) were enrolled for analyses. Significant associations with the risk of IS were detected for rs2383206 (recessive model: \( P=0.002, \ OR = 1.22, \ 95\% \ CI \ 1.08–1.38, \ I^2 = 0\%, \ FEM \) allele model: \( P=0.003, \ OR = 0.90, \ 95\% \ CI \ 0.84–0.96, \ I^2 = 0\%, \ FEM \) and rs10757274 (allele
Table 1 The characteristics of included studies for ANRIL variants and IS

| First author (year) | Country | Ethnicity | Sample size Case/Control | Genotype distribution | Minor allele (%) Case/Control | P-value for HWE | NOS score |
|---------------------|---------|-----------|--------------------------|-----------------------|-------------------------------|----------------|-----------|
| rs1333040           |         |           |                          |                       |                               |                |           |
| Akinyemi (2018) [10] | Nigeria | African   | 82/247                   | TT/TC/CC              | 42.1%/42.9%                  | NA             | 7         |
| Cao (2016) [15]     | China   | Asian     | 569/541                  | 267/247/55            | 31.4%/31.9%                  | 0.323          | 8         |
| Heckman (2013) [19] | U.S.A.  | Mixed     | 264/373                  | 93/127/44             | 40.7%/41.2%                  | 0.859          | 7         |
| Lin (2011) [23]     | Taiwan  | Asian     | 634/1352                 | 324/259/51            | 28.5%/30.8%                  | 0.829          | 7         |
| Olsson (2011) [25]  | Sweden  | Caucasian | 803/641                  | 248/392/163           | 44.7%/47.9%                  | 0.639          | 8         |
| Xiong (2018) [26]   | China   | Asian     | 200/205                  | 104/79/17             | 28.3%/26.8%                  | 0.425          | 8         |
| rs1333049           |         |           |                          |                       |                               |                |           |
| Haslacher (2016) [18]| Austria | Caucasian | 151/773                  | GG/GC/CC              | 50.3%/47.6%                  | 0.402          | 7         |
| Lin (2011) [23]     | Taiwan  | Asian     | 642/1361                 | 332/84/43             | 50.6%/47.9%                  | 0.237          | 7         |
| Xiong (2018) [26]   | China   | Asian     | 200/205                  | 104/79/17             | 46.4%/43.1%                  | 0.743          | 8         |
| rs2383206           |         |           |                          |                       |                               |                |           |
| Ding (2009) [16]    | China   | Asian     | 991/1054                 | 275/463/253           | 48.9%/45.3%                  | 0.816          | 7         |
| Hu (2009) [21]      | China   | Asian     | 352/423                  | 97/188/67             | 45.7%/41.0%                  | 0.169          | 7         |
| Xiong (2018) [26]   | China   | Asian     | 200/205                  | 104/79/17             | 48.0%/48.3%                  | 0.579          | 8         |
| Yang (2018) [28]    | China   | Asian     | 550/549                  | 162/266/122           | 45.9%/44.1%                  | 0.529          | 8         |
| rs2383207           |         |           |                          |                       |                               |                |           |
| Gschwendtner (2009) [17] | Germany | Caucasian | 962/4620                 | GG/GA/AA              | 41.1%/43.0%                  | NA             | 7         |
| Heckman (2013) [19] | U.S.A.  | Mixed     | 264/373                  | 64/131/69             | 50.9%/49.2%                  | 0.071          | 7         |
| Lin (2011) [23]     | Taiwan  | Asian     | 627/1349                 | 282/274/65            | 32.2%/35.3%                  | 0.660          | 7         |
| Xiong (2018) [26]   | China   | Asian     | 550/548                  | 236/237/77            | 35.5%/32.6%                  | 0.317          | 8         |
| Zhang (2012) [30]   | China   | Asian     | 1190/1664                | 359/569/262           | 45.9%/44.1%                  | 0.529          | 8         |
| rs10757274          |         |           |                          |                       |                               |                |           |
| Akinyemi (2018) [10] | Nigeria | African   | 82/247                   | NA/NA                 | 14.8%/13.6%                  | NA             | 7         |
| Hu (2009) [21]      | China   | Asian     | 353/430                  | 101/193/59            | 44.1%/40.7%                  | 0.579          | 7         |
| Luke (2009) [24]    | Austria | Caucasian | 503/784                  | 117/247/139           | 52.1%/46.0%                  | 0.097          | 7         |
| Xiong (2018) [26]   | China   | Asian     | 200/205                  | 92/89/19              | 31.8%/30.2%                  | 0.282          | 8         |
| Yang (2018) [28]    | China   | Asian     | 550/548                  | 236/237/77            | 35.5%/32.6%                  | 0.317          | 8         |
| Zhang (2012) [30]   | China   | Asian     | 1190/1664                | 359/569/262           | 45.9%/44.1%                  | 0.529          | 8         |
| rs10757278          |         |           |                          |                       |                               |                |           |
| Akinyemi (2018) [10] | Nigeria | African   | 82/247                   | NA/NA                 | 11.0%/12.6%                  | NA             | 7         |
| Bi (2015) [14]      | China   | Asian     | 116/118                  | 38/49/29              | 46.1%/32.6%                  | 0.307          | 8         |
| Ding (2009) [16]    | China   | Asian     | 999/1055                 | 378/431/190           | 40.6%/40.0%                  | 0.538          | 8         |
| Gschwendtner (2009) [17] | Germany | Caucasian | 952/2462                | NA/NA                 | 50.5%/46.9%                  | NA             | 7         |
| Heckman (2013) [19] | U.S.A.  | Mixed     | 263/374                  | 78/139/48             | 43.9%/47.1%                  | 0.705          | 7         |
| Helgadottir (2008) [20] | New Zealand | Caucasian | 705/15012               | NA/NA                 | 46.8%/43.3%                  | NA             | 7         |
| Lemmens (2009) [22] | Belgium | Caucasian | 914/809                  | 176/461/277           | 55.5%/48.1%                  | 0.207          | 8         |
| Olsson (2011) [25]  | Sweden  | Caucasian | 834/665                  | 222/415/197           | 48.5%/45.6%                  | 0.343          | 8         |
| Xiong (2018) [26]   | China   | Asian     | 200/205                  | 53/95/52              | 49.8%/47.1%                  | 0.656          | 8         |
| Zhang (2012) [30]   | China   | Asian     | 986/1452                 | 302/448/236           | 46.7%/49.2%                  | 0.298          | 8         |

Abbreviation: NA, Not available.
model: \( P=0.006, \ OR = 0.91, \ 95\% CI \ 0.86-0.97, \ I^2 = 25\%, \ \text{FEM} \) variants in overall analyses. Further subgroup analyses by ethnicity of participants revealed that rs2383206, rs10757274 and rs10757278 variants were all significantly correlated with the risk of IS in Asians. Moreover, rs10757278 polymorphism was also significantly correlated with the risk of IS in Caucasians (see Table 2 and Supplementary Figure S1).

**Sensitivity analyses**
We performed sensitivity analyses to examine whether studies that deviated from HWE would impact the results of synthetic analyses. No alterations of results were detected in sensitivity analyses when we omitted one specific study each time, which suggested that our pooled results were statistically stable and reliable.
Table 2 Overall and subgroup analyses for ANRIL variants and IS

| Polymorphisms | Population | Sample size | Dominant comparison | Recessive comparison | Additive comparison | Allele comparison |
|---------------|------------|-------------|---------------------|----------------------|---------------------|------------------|
|               |            |             |  \(P\) value | OR (95%CI) |  \(P\) value | OR (95%CI) |  \(P\) value | OR (95%CI) |  \(P\) value | OR (95%CI) |
| rs1333040     | Overall    | 2552/3359   | 0.22 | 1.07 (0.96–1.20) | 0.07 | 0.86 (0.73–1.01) | 0.98 | 1.00 (0.90–1.12) | 0.07 | 1.08 (0.99–1.16) |
|               | Asian      | 1403/2098   | 0.50 | 1.06 (0.91–1.20) | 0.21 | 0.86 (0.73–1.01) | 0.95 | 1.00 (0.90–1.12) | 0.28 | 1.06 (0.95–1.18) |
| rs1333049     | Overall    | 1543/2888   | 0.10 | 0.89 (0.77–0.92) | 0.20 | 1.13 (0.96–1.34) | 0.68 | 1.02 (0.91–1.17) | 0.07 | 0.92 (0.84–1.01) |
|               | Asian      | 1392/2115   | 0.25 | 0.91 (0.79–1.06) | 0.13 | 1.13 (0.96–1.34) | 0.84 | 0.99 (0.86–1.13) | 0.10 | 0.92 (0.84–1.02) |
| rs2383206     | Overall    | 2987/3600   | 0.05 | 0.90 (0.80–1.00) | 0.002 | 1.22 (1.08–1.38) | 0.98 | 1.00 (0.83–1.20) | 0.003 | 0.90 (0.84–0.96) |
|               | Asian      | 2733/3346   | 0.05 | 0.90 (0.80–1.00) | 0.002 | 1.22 (1.08–1.38) | 0.98 | 1.00 (0.83–1.20) | 0.002 | 0.89 (0.83–0.96) |
| rs2383207     | Overall    | 3793/8372   | 0.64 | 1.02 (0.93–1.13) | 0.79 | 0.98 (0.85–1.13) | 0.79 | 0.99 (0.90–1.09) | 0.21 | 1.04 (0.98–1.10) |
|               | Asian      | 2567/3739   | 0.43 | 1.04 (0.94–1.16) | 0.99 | 1.00 (0.97–1.32) | 0.53 | 0.97 (0.87–1.07) | 0.48 | 1.03 (0.95–1.11) |
| rs10757274    | Overall    | 3106/16440  | 0.07 | 0.91 (0.82–1.01) | 0.26 | 1.14 (0.91–1.44) | 0.95 | 1.01 (0.94–1.20) | 0.006 | 0.91 (0.86–0.97) |
|               | Caucasian  | 809/10359   | 0.11 | 0.86 (0.71–1.03) | 0.80 | 1.10 (0.54–2.11) | 0.81 | 1.05 (0.71–1.53) | 0.55 | 0.91 (0.67–1.23) |
|               | Asian      | 1743/2299   | 0.11 | 0.89 (0.78–1.03) | 0.007 | 1.23 (1.06–1.44) | 0.80 | 1.04 (0.76–1.42) | 0.009 | 0.89 (0.81–0.97) |
| rs10757278    | Overall    | 6051/24199  | 0.35 | 0.90 (0.72–1.12) | 0.10 | 1.16 (0.97–1.37) | 0.20 | 0.95 (0.87–1.03) | 0.05 | 0.91 (0.82–1.00) |
|               | Caucasian  | 3405/20748  | 0.12 | 0.74 (0.51–1.08) | 0.001 | 1.31 (1.12–1.54) | 0.70 | 1.03 (0.89–1.18) | &lt;0.001 | 0.85 (0.80–0.90) |
|               | Asian      | 2301/2830   | 0.89 | 0.98 (0.77–1.26) | 0.22 | 1.17 (0.91–1.60) | 0.03 | 0.88 (0.76–0.99) | 0.40 | 0.92 (0.76–1.11) |

Abbreviation: NA, Not available. The values in bold represent that there are statistically significant differences between cases and controls. All investigated ANRIL variants contain a major allele (M) and a minor allele (m). In the current meta-analysis, dominant model is defined as MM versus Mm + mm, recessive model is defined as mm versus MM + Mm, Additive model is defined as Mm versus MM + mm, and the allele model is defined as M versus m.

Publication biases
Funnel plots were used to estimate publication biases. We did not find obvious asymmetry of funnel plots in any comparisons, which suggested that our findings were unlikely to be influenced by severe publication biases (see Supplementary Figure S2).

Discussion
To the best of our knowledge, this is the most comprehensive meta-analysis on associations between ANRIL variants and the risk of IS. Our overall and subgroup analyses suggested that rs2383206, rs10757274 and rs10757278 variants were all significantly associated with an increased risk of IS in Asians. In addition, rs10757278 polymorphism was also significantly associated with an increased risk of IS in Caucasians. As shown in Supplementary Figure S1, for rs1333040, rs1333049, rs2383206 and rs2383207 variants, between-study heterogeneities were trivial, and thus pooled analyses were mainly performed with FEM. For rs10757274 and rs10757278 variants, however, obvious between-study heterogeneities were observed for recessive and additive comparisons, and thus REMs were employed for these analyses.

There are several points that need to be addressed about this meta-analysis. First, the exact function of ANRIL is still unclear, and therefore the underlying mechanisms of our positive findings need to be investigated by future investigations. Second, the pathogenic mechanism of IS is rather complex, and it is unlikely that a single genetic variant can significantly contribute to its development. So to better illustrate potential correlations of certain genetic variants...
with IS, we strongly recommend further studies to perform haplotype analyses and explore potential gene–gene interactions. Third, it is also worth noting that according to our findings, the associations between ANRIL variants and IS may be ethnic-specific, and this may explain why inconsistent results were observed in included original studies, especially when these studies were performed in different populations.

As with all meta-analysis, the present study certainly has some limitations. First, our results were derived from unadjusted analyses, and lack of further adjusted analyses for age, gender, smoking status and co-morbidity conditions (such as hypertension, diabetes, dyslipidemia, coronary artery disease and peripheral artery disease) may impact the reliability of our findings since the above-mentioned variables may also impact the individual susceptibility to IS [31,32]. Second, obvious heterogeneities were still found in several subgroup comparisons for rs10757274 and rs10757278 variants, which indicated that the controversial results of included studies could not be fully explained by differences in ethnic background, and other baseline characteristics of participants may also contribute to between-study heterogeneities [33,34]. Third, associations between ANRIL variants and IS may also be modified by gene–environment interactions. However, most eligible studies ignore these potential interactions, which impeded us to perform relevant analyses accordingly [35,36]. On account of above-mentioned limitations, our findings should be cautiously interpreted.

Conclusions
In conclusion, our meta-analysis suggested that rs2383206, rs10757274 and rs10757278 variants may impact individual susceptibility to IS in Asians. Moreover, rs10757278 polymorphism may also impact individual susceptibility to IS in Caucasians. However, considering that the sample sizes of several comparisons were still relatively small, further well-designed studies with larger sample sizes are still warranted to confirm our findings.

Author Contribution
Cheng Tan and Shoujun Yang conceived the study and participated in its design. Cheng Tan and Junzhi Liu conducted the systematic literature review. Jun Wei performed data analyses. Cheng Tan and Shoujun Yang drafted the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Abbreviations
ANRIL, antisense non-coding RNA in the INK4 locus; CI, confidence interval; FEM, fixed-effect model; HWE, Hardy–Weinberg equilibrium; IS, ischemic stroke; NOS, Newcastle–Ottawa scale; OR, odds ratio; REM, random-effect model.

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