Apolipoprotein E Epsilon 4 Enhances the Association between the rs2910164 Polymorphism of miR-146a and Risk of Atherosclerotic Cerebral Infarction

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Aim: To analyse the relationship between two potentially functional single-nucleotide polymorphisms (SNPs) of the miR-146a gene (rs2910164 and rs57095329) and the risk of atherosclerotic cerebral infarction (ACI).

Methods: A total of 297 patients with ACI and 300 matched healthy individuals were enrolled in the study. The miR-146a polymorphism was detected using the polymerase chain reaction—restriction fragment length polymorphism method.

Results: A significant difference in the C allele frequency at rs2910164 (p=0.028) was noted between patients with ACI and control subjects. In contrast, the genotype and allele frequencies of rs57095329 were not statistically associated with ACI. In addition, the decreased expression of miR-146a was significantly more frequent in ACI patients who were ApoE⁴ carriers (p=0.0233), and rs2910164 G>C was intimately associated with the ApoE⁴-containing genotype in patients compared with the ApoE⁴ carriers (p=0.0323).

Conclusions: Our findings indicated that the C allele of rs2910164 miR-146a is an important risk factor for ACI, and ApoE⁴ may function through attenuating miR-146a expression to enhance ACI susceptibility. This study provides new information about the possible relationship between miR-146a and ApoE⁴ in the development of ACI, with potentially important therapeutic implications.

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Key words: Polymorphism, microRNA-146a, Apolipoprotein E, Atherosclerotic cerebral infarction

Introduction

Atherosclerotic cerebral infarction (ACI) is a complex disease caused by the combination of multiple risk factors, such as atherosclerosis, hypertension, diabetes, and dyslipidaemia, among which atherosclerosis is considered as the main factor involved in the pathogenesis of ACI¹. Atherosclerosis is characterized by chronic local inflammation of the vascular wall, resulting in the accumulation of lipids and macrophage-derived foam cells in the subendothelial space². Although the inflammatory nature of atherosclerosis is widely accepted, the complex mechanisms underlying the atherogenic proinflammatory processes remain controversial. Recent findings have suggested that ACI
has a significant genetic component\(^3,\)\(^4\). Considerable evidence gathered to date has allowed us to identify at least five potential important pathways that may be related to the genetic risk factors of atherothrombosis, including lipoprotein metabolism, inflammation, the renin-angiotensin-aldosterone system (RAAS), platelet biology and function, and blood coagulation and fibrinolysis, contributing to the risk of ACI\(^5\).

MicroRNAs (miRNAs) are small noncoding RNAs that are 20–23 nucleotides in length and regulate gene expression in a sequence-specific manner\(^6\). Accumulating evidence has implicated miRNAs as essential regulators of atherosclerosis by targeting important factors or key pathways\(^7,\)\(^8\). Abnormal miRNA expression has also been observed in various diseases that are associated with inflammatory and immune processes\(^9-11\). MiR-146a is recognized for its ability to silence multiple inflammatory targets, including IRAK1 and TRAF6, and suppress TLR-driven NF-\(\kappa\)B signaling in macrophages and other haematopoietic cells. Reduction of miR-146a levels in these cell lines could enhance the susceptibility to atherosclerosis and sepsis\(^12-14\). The current evidence supports the hypothesis that miR-146a operates as an important epigenetic regulator in various pathways involved in atherosclerosis and stroke. Thus, we were prompted to explore the relationship between miR-146a and ACI\(^15,\)\(^16\). Certain single-nucleotide polymorphisms (SNPs) could influence the function of the target genes and are associated with diseases risk\(^17\). Two SNPs in the miR-146a gene, rs2910164 and rs57095329, have functional importance in modifying the expression of mature miR-146a and have been reported to be associated with several inflammation-associated diseases\(^18-21\).

Apolipoprotein E (ApoE) plays an important role in lipid metabolism and cholesterol transport, which are considered to be candidate pathways with antiatherogenic functions\(^22,\)\(^23\). Several studies have reported that ApoE polymorphisms serve as a major genetic risk factor for a wide spectrum of inflammatory metabolic diseases, including atherosclerosis, cerebrovascular disorders, and hyperlipoproteinemia, and they are also well recognized for their ability to suppress atherosclerosis\(^24-26\). Interestingly, it has been recently reported that ApoE regulates the expression of the anti-inflammatory microRNA miR-146a in monocytes and macrophages\(^27\). To the best of our knowledge, no study to date has examined the association of miR-146a polymorphisms with the risk of ACI. In this study, we conducted an association analysis to ascertain whether the two functional polymorphisms of miR-146a contribute to the risk of ACI and further studied the distribution of ApoE genotypes in these study populations.

### Methods

#### Study Population

Our study consecutively recruited 297 patients with ACI (120 women and 177 men; mean age = 62.6 ± 8.63 years) from the Department of Cardiovascular Internal Medicine and the Department of Neurorsurgery of the Affiliated second Hospital of Guangdong Medical University and 300 healthy controls (130 women and 170 men; mean age = 61.1 ± 9.58 years) from the medical examination center of the Affiliated Hospital of Guangdong Medical University. Patient diagnoses were verified with either computed tomography (CT) or magnetic resonance imaging (MRI), and all the patients with ACI were classified into subtypes according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification\(^27\). All subjects enrolled in our study were Chinese Han with similar dietary pattern, and the patients with histories of ischemic cerebrovascular diseases, cardiogenic cerebral infarctions, cerebral hemorrhage, coronary artery diseases (CAD), autoimmune diseases, systemic inflammatory diseases, blood diseases, and malignant tumors were excluded from the study. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki, written informed consent was obtained from all the enrolled participants, and this study was approved by the Ethics Committee of the Guangdong Medical University.

#### DNA Isolation and Genotyping

Genomic DNA was isolated from peripheral blood samples using a blood genomic DNA extraction kit (Tiangen, China). The DNA was genotyped using the ABI PRISM SNapShot method (Applied Biosystems, Foster, CA). The polymerase chain reaction (PCR) primers used for the rs2910164 polymorphism were 5′-GAAGTGGCCATGCTCGTGT-3′ and 5′-TCAATTGGCAGCCTCAGG-3′. The primers used for rs57095329 polymorphism were 5′-AGCCAATGGGATTCAAG-3′ and 5′-AGGAGTTCCTGTTTCAGCG-3′. In brief, the SNapShot reaction was performed in a 10-ml final volume containing the SNapShot Multiplex Ready Mix (5 ml), primer mix (0.02–0.6 mmol/L), and templates (4 ml) consisting of the multiplex PCR products, which were purified using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). The cycling program included 25 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 40 s. Extension products were purified by a 15-min incu-
nuclear cells (PBMCs) were isolated using the density isolation of mononuclear cells. Peripheral blood mononuclear cells (PBMCs) were pelleted and lysed using TRizol. The lysate was centrifuged at 13,400 rpm. The integrity of RNA from all samples was further purified by performing the washing steps and then eluting the RNA from the membrane in water. Next, the integrity of the RNA samples was verified by agarose gel electrophoresis and stored at −80°C.

**Real-Time PCR**

MiR-146a expression was analyzed using the miScript SYBR Green PCR kit (Qiagen, Germany) according to the manufacturer’s instructions (Qiagen, Germany). The assay was performed in triplicate. Human U6 was used as the internal control. The comparative threshold cycle (Ct) method was used to assess the relative changes in expression. In brief, \(2^{\Delta\Delta\text{Ct}}\) represents the fold change in miR-146a expression between sample groups. A no-template control was run with the positive samples to assess the overall specificity of the reaction \(10\). The expression levels of IRAK-1 were analyzed using the miScript SYBR Green PCR kit. The IRAK-1 expression levels were analyzed in triplicate, and the expression was normalized to the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which was used as an internal control. The expression levels of IRAK-1 were also calculated using the \(2^{\Delta\Delta\text{Ct}}\) method.

**Plasma Lipid Measurements**

Blood samples for lipid measurements were withdrawn from subjects after an overnight fast. The plasma triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol levels were measured using a Hitachi 7600 automatic analyser (Hitachi Instruments Corporation, Tokyo, Japan).

**Statistical Analyses**

Statistical analyses were performed using SPSS software, version 19.0 (IBM, Armonk, NY, USA). These data are presented as percentage frequencies or means ± standard deviation (SD). Allele frequencies were calculated from the genotypes of all subjects. The allele and genotype frequencies of miR-146a between the patients and control subjects were compared using Fisher’s exact test or chi-squared test. Hardy–Weinberg equilibrium (HWE) was assessed using HWE software. Risk factors were screened using Student’s \(t\)-test or chi-squared test. Analyses were repeated in subgroups stratified by miR-146a and ApoE4 carrier status. Associations were expressed as odds ratios (ORs) or risk estimates with 95% confidence intervals (CIs). The relationships between different genotypes of miR-146a and ACI were evaluated using analysis of variance (ANOVA). A \(p\) value < 0.05 was considered to be statistically significant. Power analysis was performed using QUANTO 1.2 software.
**Table 1.** Characteristic of Controls and ACI Patients

| Clinic data          | ACI patients (297) | Controls (300) | P value |
|----------------------|-------------------|----------------|---------|
| Mean ages (years)    | 62.6 ± 8.63       | 61.1 ± 9.58    | 0.064   |
| Male/Female          | 177/120           | 170/130        | 0.468   |
| BMI (kg/m²)          | 22.6 ± 3.41       | 23.35 ± 2.51   | 0.754   |
| History of hypertension, n (%) | 197 (66.33%) | 66 (22.00%) | <0.001 * |
| History of diabetes, n (%) | 62 (20.86%)  | 11 (3.67%)    | <0.001 * |
| HDL (mmol/L)         | 1.19 ± 0.47       | 1.25 ± 0.31    | 0.015 * |
| LDL (mmol/L)         | 2.85 ± 1.06       | 2.91 ± 0.86    | 0.043 * |
| Total Cholesterol (mmol/L) | 4.91 ± 0.97   | 4.59 ± 0.62    | 0.028 * |
| Total Cholesterol (mmol/L) | 1.84 ± 1.04   | 1.62 ± 0.63    | 0.075   |
| ApoE ε4, n (%)       | 94 (31.65%)       | 37 (12.33%)    | <0.001 * |

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein. *p < 0.05.

**Table 2.** Genotype and allele Distributions of the miR-146a polymorphisms between controls and ACI Patients

| rs2910164 G > C | ACI patients (297), n (%) | Controls (300) n (%) | OR (95% CI) | P-value |
|-----------------|---------------------------|----------------------|-------------|---------|
| CC              | 141 (47.47%)              | 113 (37.67%)         |             | 0.052   |
| GC              | 128 (43.10%)              | 152 (50.66%)         |             |         |
| GG              | 28 (9.43%)                | 35 (11.67%)          |             |         |
| GC/GG           | 156 (52.52%)              | 187 (62.33%)         | 0.667 (0.482-0.927) | 0.015 * |
| GC/CC           | 269 (90.57%)              | 265 (88.33%)         | 1.27 (0.750-2.15) | 0.373   |
| C alleles       | 410 (69.02%)              | 378 (63.00%)         | 0.764 (0.601-0.972) | 0.028 * |
| G alleles       | 184 (30.98%)              | 222 (37.00%)         |             |         |

| rs57095329 G > A | ACI patients (297), n (%) | Controls (300) n (%) | OR (95% CI) | P-value |
|-----------------|---------------------------|----------------------|-------------|---------|
| AA              | 200 (67.34%)              | 211 (70.33%)         |             | 0.620   |
| GA              | 87 (29.29%)               | 82 (27.33%)          |             |         |
| GG              | 10 (3.37%)                | 7 (2.33%)            |             |         |
| GA/GG           | 97 (32.66%)               | 89 (29.66%)          | 1.15 (0.813-1.63) | 0.480   |
| GA/AA           | 287 (95.63%)              | 293 (97.67%)         | 0.686 (0.257-1.83) | 0.472   |
| A alleles       | 487 (81.99%)              | 504 (84.00%)         | 1.153 (0.852-1.561) | 0.354   |
| G alleles       | 107 (18.01%)              | 96 (16.00%)          |             |         |

Adjusted for age, sex, hypertension and diabetes. *p < 0.05.

**Results**

**Clinical Characteristics**

The clinical characteristics of all the participants in the study are summarized in Table 1. Of the 597 participants, 297 were ACI patients and 300 were healthy controls. The mean age was 62.6 (± 8.63) years for the ACI patients and 61.1 (± 9.58) years for the control subjects, and the gender (male-to-female) ratio was 1:1.48 in the case group and 1:1.31 in the control group. No statistically significant difference was noted between the patients and controls in terms of sex (p = 0.468) or age (p = 0.064). The risk factors examined (e.g., hypertension and diabetes) were significantly more common in the ACI group compared with the control group. Moreover, compared with the controls, the patients with ACI exhibited increased TC (p = 0.028), HDL (p = 0.015), and LDL (p = 0.043). Individuals with hypertension (p < 0.001) and diabetes (p < 0.001) exhibited increased risk of ACI. However, patients with ACI exhibited a trend of increased total TG compared with controls, but no significant difference was noted (p = 0.075). Moreover, as expected, ApoE ε4 allele frequencies were significantly elevated in ACI patients compared with controls (p < 0.001). Power analysis revealed that, with our sample size, we would have 93.2% power for rs2910164 and 78.1% power for rs57095329 to detect a genotype
relative risk with an OR of 1.5 at a significance level of 0.05.

**MiR-146a Polymorphisms in ACI Patients and Controls**

All the enrolled participants were successfully genotyped for the rs2910164 and rs57095329 SNPs. All genotype distributions were in HWE in the ACI patients and controls (Supplement Table 1). The genotype and allele frequencies of miR-146a polymorphisms in the study are presented in Table 2. Although a trend was noted, no statistical association with the genotype frequencies of the rs2910164 SNP with ACI patients and controls was observed ($p=0.052$). In contrast, a significant difference in the C allele frequency of the rs2910164 SNP was noted ($p=0.028$). Moreover, a significant difference was observed in the ACI patients compared with the controls in a recessive model (GG + GC vs. CC) ($p=0.015$), which indicated that the rs2910164 polymorphism is a risk factor for ACI (Table 2). Regarding the rs57095329 polymorphism, no statistically significant difference was observed between the ACI patients and healthy controls for either the genotype or allele frequencies of the rs57095329 SNP (genotype frequencies $p=0.620$, allele frequencies $p=0.354$).

**MiR-146a Polymorphisms in ApoE Genotype Groups in ACI Patients and Controls**

The prevalence of the ApoE4-containing phenotypes was significantly increased in individuals with cardiovascular and cerebrovascular ailments, such as myocardial infarction, hypertension, coronary heart disease, and stroke. We examined whether a significant association exists between miR-146a and ApoE4 isoforms in ACI patients. As shown in Table 3, when these data were stratified for the presence or absence of the E4 isoform, the C allele of rs2910164 remained significantly different between ACI patients and controls in the ApoE4(+) subgroup ($p=0.033$) but not in the ApoE4(−) groups ($p=0.472$). In the recessive model (GG + GC vs. CC), a significant difference was observed in ApoE4(+) patients compared with ApoE4(+) controls ($p=0.04$). However, no significant difference was detected in the ApoE4(−) subgroups ($p=0.440$). Besides, neither the genotype nor the allele frequencies in rs57095329 exhibited significant differences in the ApoE4 mutation subgroups [ApoE4(+) genotype and allele frequencies: $p=0.326$ and $p=0.492$, respectively; ApoE4(−) genotype and allele frequencies: $p=0.222$ and $p=0.146$, respectively] (Table 3).

### Table 3. Genotype and allele distributions in ApoE genotype groups with ACI patients and controls

| rs2910164 (n) | Genotypes n (%) | genotype frequencies |
|---------------|------------------|----------------------|
| ApoE4(+)      |                  |                      |
| ACI (94)      | 59 (62.77%)      | 28 (29.79%)          |
| Controls (37) | 16 (43.24%)      | 16 (43.24%)          |
| ApoE4(−)      |                  |                      |
| ACI (203)     | 82 (40.39%)      | 100 (49.26%)         |
| Controls (263)| 97 (36.88%)      | 136 (51.71%)         |
| Rs57095329 (n) |                 |                      |
| ApoE4(+)      |                  |                      |
| ACI (94)      | 66 (70.21%)      | 27 (28.72%)          |
| Controls (37) | 25 (67.57%)      | 10 (27.03%)          |
| ApoE4(−)      |                  |                      |
| ACI (203)     | 134 (66.01%)     | 60 (29.56%)          |
| Controls (263)| 186 (70.72%)     | 72 (27.38%)          |

### Adjusted for age, sex, hypertension and diabetes. *$p<0.05$. 

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The Influence of ApoE<sub>4</sub> on miR-146a Expression in ACI Patients

From the case-control study above, we found that rs2910164 of miR-146a exhibited a high-risk genotype in ApoE<sub>4</sub> patients with ACI. To further explore the influence of ApoE<sub>4</sub> on the expression of mature miR-146a, we examined the expression levels of the rs2910164 miR-146a genotypes in PBMCs that were obtained from the ACI patients who were E4 allele carriers. According to the data obtained, the mean level of miR-146a mRNA expression was significantly reduced in ApoE<sub>4</sub>(+) patients with ACI compared with ApoE<sub>4</sub>(-) patients (p = 0.0233), and the mean level of miR-146a mRNA was also significantly reduced in ApoE<sub>4</sub>(+) healthy control compared with ApoE<sub>4</sub>(-) control (p = 0.039) (Fig. 1A). Moreover, regarding ACI patients with ApoE<sub>4</sub>(+), individuals with the CC genotype for rs2910164 exhibited significantly lower levels of miR-146a than those with the GG or GC genotype (p = 0.0323) (Fig. 1B). However, the mean level of miR-146a revealed no significant difference between the CC and GG + GC genotypes for rs2910164 in ACI patients who were ApoE<sub>4</sub>(-) carriers, suggesting that the CC genotype for rs2910164 has a stronger influence on mature miR-146a expression in ACI patients with ApoE<sub>4</sub>(+) but not ApoE<sub>4</sub> (-). Furthermore, patients with the CC genotype expressed significantly higher relative expression of IRAK-1 in ApoE<sub>4</sub>(+) patients (p = 0.048; compared with GG/GC, Fig. 1C); however, no statistically significant difference was observed in ApoE<sub>4</sub>(-) subjects. The comparison of genotype distributions between the ACI subjects revealed a statistical association between the rs2910164 polymorphism of miR-146a and risk of ACI. Furthermore, ApoE<sub>4</sub> may alter the effect of C allele mutations in rs2910164 on decreasing miR-146a expression, further contributing to ACI susceptibility.

To further explore whether rs2910164 SNP may influence lipid metabolism and cholesterol transport that has been proved to be associated with ApoE, we also evaluated the possible association between the rs2910164 SNP of miR-146a and TC/total TG levels in patients with or without ApoE<sub>4</sub>(+). However, neither the TC nor the total TG levels exhibited a statistically significant difference between the ApoE<sub>4</sub>(+) and ApoE<sub>4</sub>(-) groups. In addition, no significant difference was observed between the CC and GG + GC genotypes for rs2910164 miR-146a (Fig. 2).

Discussion

MiR-146a is a powerful innate immune and pro-inflammatory-related regulator of immune and inflammatory responses. MiR-146a drives PBMCs toward Th1 polarization, and the levels of this particular miRNA are increased in patients with diseases such as acute coronary syndrome<sup>28</sup>. MiR-146a is upregulated in THP-1 cells, macro-phages, Langerhans cells, and monocytes after induction by lipopolysaccharide (LPS), TNF-α, IL-1β, or TGF-β1<sup>29, 30</sup>. Furthermore, miR-146a is a novel regulator of vascular smooth muscle cell (VSMC) fate, which is crucial in the pathogenesis of proliferative cardiovasc-ular diseases, including atherosclerosis and post-angioplasty restenosis<sup>31</sup>. These data suggest that miR-146a is associated with the development of atherosclerosis, which is one of the important risk factors for ACI<sup>32, 33</sup>. However, few studies to date have examined the relationship between miR-146a and ACI. Here we focused on identifying the possible association between miR-146a functional polymorphisms and other risk factors of ACI in a...
SNPs present in precursor and mature miRNAs influence the levels of mature miRNAs and are associated with various diseases\cite{34-36}. In our study, two functional SNPs in miR-146a were chosen to evaluate the association between miR-146a polymorphisms and ACI risk. The two SNPs in miRNA-146a, rs2910164 and rs57095329, have been reported to influence the expression level of mature miR-146a; thus, these SNPs may ultimately affect an individual's susceptibility to disease\cite{10, 37, 38}. Our results revealed that the rs2910164 polymorphism was associated with an increased risk of ACI; the C allele of rs2910164 was more frequent in patients with ACI compared with the G allele, indicating that the C allele of rs2910164 is associated with an increased risk of ACI susceptibility. The SNP rs2910164 G/C is located in the stem region, and the C allele creates a mismatch in the stem structure of pre-miR-146a. This mismatch results in a decrease in the total levels of mature miR-146a and influences the transcription of target genes, ultimately leading to disease pathogenesis\cite{17, 18}. MiR-146a is assumed to be protective against atherosclerosis given its ability to suppress NF-κB signaling, the TLR4 signaling pathway, and the apparent activation of endothelial cells. miR-146a levels were elevated in human atherosclerotic plaques as well as in the circulation and plaques of atherosclerotic mice because of the strong activation of NF-κB signaling in plaques, implying that miR-146a is induced as part of a negative feedback loop\cite{32, 39, 40}. Xiong et al. stated that the GC and CC genotypes of the miRNA-146a rs2910164 polymorphism are associated with an increased risk of CAD\cite{41}, whose risk subsequently increases with the presence of metabolic syndrome induced by inflammation\cite{42, 43}. In this study, we confirmed that patients with reduced miR-146a expression exhibit an increased risk of ACI. In this study, significantly reduced miR-146a mRNA levels were observed in ACI subjects. Considering the supporting evidence that the decreased miR-146a levels are also associated with the development of cardiovascular diseases\cite{32, 33, 41}, we speculate that the C allele of rs2910164 may reduce miR-146a expression and subsequently weaken anti-inflammatory action in the pathogenesis of ACI, thus contributing to the pathological process and increased risk of ACI.

Another novel finding of this study was that ACI patients with the ApoE4 allele exhibited reduced miR-146a expression compared with controls. More interestingly, CC genotype carriers exhibited reduced miR-146a expression compared with GG or GC genotype carriers. The ApoE4(+) group contained 2.5-fold more ACI patients than controls, whereas the ApoE4(−) group included 1.4-fold more controls than ACI patients. It appears that carrying the E4 allele attenuates the level of miR-146a, which increases the risk of ACI. Moreover, the CC genotype of the miRNA-146a rs2910164 polymorphism exhibited a more significant effect in the ApoE4(+) group compared with the ApoE4(−) group. ApoE is an important regulator of plasma lipoprotein metabolism and cholesterol homeostasis, and variants of ApoE are major genetic risk factors for a wide spectrum of inflammatory metabolic diseases\cite{44, 45}. Individuals who carry at least one copy of the ApoE4 allele have an increased risk of developing atherosclerosis, which is an accumulation of fatty deposits and scar-like tissue in the lining of the arteries that narrow the arteries, thus
increasing the risk of ACI. \(^{46}\) MiR-146a has been reported to negatively regulate multiple inflammatory targets, including IRAK1 and TRAF6, and to suppress NF-\(\kappa\)B-driven TNF-\(\alpha\) expression. \(^{47, 48}\) As shown in our results, the reduction of miR-146a expression increased IRAK1 levels significantly in CC genotypes in ApoE \(e4(+)\) carriers. Reduction of miR-146a expression can reverse the effect of NF-\(\kappa\)B activity inhibition. \(^{49}\) ApoE controls inflammation by suppressing inflammatory NF-\(\kappa\)B signaling. \(^{24}\) Mechanistically, ApoE increases the expression of the transcription factor PU.1, which increases miR-146 levels to suppress NF-\(\kappa\)B signaling. Li et al. demonstrated that ApoE regulates the expression of miR-146a in monocytes and macrophages to repress NF-\(\kappa\)B signaling in these cells, and intravascular delivery of miR-146a mimetics can inhibit atherogenesis in mouse models. \(^{15}\) However, the \(e4\) gene polymorphism causes strong dysfunction of ApoE, which suppresses the level of miR-146a. \(^{33, 38, 50}\) Based on the finding that ApoE \(e4\) induced decreasing expression of miR-146a in ACI patients, we suggest that the downregulation of NF-\(\kappa\)B-dependent pathways by miR-146a through a critical negative feedback regulatory loop was attenuated by the effects of ApoE \(e4\). Furthermore, considering the important roles of ApoE in lipid and cholesterol metabolism, we explored whether rs2910164 influences TC and TT levels. Although no significant association was observed, we speculate that the rs2910164 polymorphism, with its synergistic effect with ApoE, may contribute to the risk of ACI but not through the lipid and cholesterol pathway. Nevertheless, the mechanisms of the interaction of ApoE \(e4\) dysfunction with the CC genotype or C allele of rs2910164 should be further examined.

**Conclusions**

This is the first study to identify a significant association between the rs2910164 polymorphism of miR-146a and risk of ACI in the south Chinese Han population. The C allele of rs2910164 was associated with the E4-containing phenotype of ApoE, which plays an important role in enhancing ACI susceptibility. This study provides new information about the relationship between miR-146a and ApoE in the development of ACI. However, more work is still required to shed light on the role of miR-146a and ApoE in the pathogenesis of ACI and further clarify its prognostic and therapeutic potential.

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Supplement Table 1.
The Hardy-Weinberg equilibrium assay for rs653765 and rs514049 genotypes in the ACI cases and controls

|               | Rs2910164 |         |         | P-value |
|---------------|-----------|---------|---------|---------|
|               | CC        | GC      | GG      |         |
| ACI patients  | 141       | 128     | 28      | 0.892   |
| Control       | 113       | 152     | 35      | 0.133   |
| Rs57095329    | GG        | GA      | AA      |         |
| ACI patients  | 200       | 87      | 10      | 0.887   |
| Control       | 211       | 82      | 7       | 0.770   |

|               | Rs2910164 |         |         |         |
|---------------|-----------|---------|---------|---------|
|               | CC        | GC      | GG      |         |
| ACI patients  | 59        | 28      | 7       | 0.170   |
| Control       | 16        | 28      | 7       | 0.755   |
| Rs57095329    | GG        | GA      | AA      |         |
| ACI patients  | 66        | 27      | 1       | 0.328   |
| Control       | 25        | 27      | 2       | 0.469   |

|               | Rs2910164 |         |         |         |
|---------------|-----------|---------|---------|---------|
|               | CC        | GC      | GG      |         |
| ACI patients  | 82        | 100     | 21      | 0.237   |
| Control       | 97        | 136     | 30      | 0.086   |
| Rs57095329    | GG        | GA      | AA      |         |
| ACI patients  | 134       | 60      | 9       | 0.495   |
| Control       | 186       | 72      | 5       | 0.514   |