Association of miR-34a, miR-130a, miR-150 and miR-155 polymorphisms with the risk of ischemic stroke

GUN HO CHO1*, KI HAN KO1*, JUNG OH KIM1, JINKWON KIM2, SEUNG HUN OH2, IN BO HAN3, KYUNG GI CHO3, OK JOON KIM2, JINKUN BAE3 and NAM KEUN KIM1

1Department of Biomedical Science, College of Life Science, CHA University, Seongnam 463-400; Departments of 2Neurology, 3Neurosurgery and 4Emergency Medicine, CHA Bundang Medical Center, School of Medicine, CHA University, Seongnam 463-712, Republic of Korea

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Abstract. MicroRNAs (miRNAs or miRs) are small (19-23 nt) non-coding RNA molecules that are endogenous regulators of gene expression. Previous studies have found that some miRNAs are related to the progression of ischemia in the cerebral artery. Furthermore, a recent study found a significant association between miRNA single nucleotide polymorphisms (SNPs) and the risk of ischemic stroke. Therefore, it may be valuable to investigate associations between megakaryocyte formation-related miRNA polymorphisms and the prevalence of ischemic stroke. We thus conducted a case-control study of 1,000 individuals who were screened for 4 miRNA polymorphisms (miR-34a rs6577555C>A, miR-130a rs731384C>T, miR-150 rs73056039G>A and miR-155 rs767649T>A) by PCR-RFLP analysis. The study population comprised 596 patients with ischemic stroke and 404 control subjects without any history of neurological disorders. We observed associations between miRNA polymorphisms and individual stroke subtypes. The miR-150 polymorphisms were significantly associated with ischemic stroke subgroups, such as left anterior descending artery (LAD) disease [GG vs. AA: adjusted odds ratio (AOR), 1.922; 95% confidence interval (CI), 1.003-3.681] and cardioembolism (GG vs. AA: AOR, 2.996; 95% CI, 1.293-6.939). Additionally, Cox proportional analysis indicated that the miR-150GA genotype was associated with survival in patients with ischemic stroke [adjusted hazard ratio (HR), 2.063; 95% CI, 1.142-3.727; P=0.017] and with the LAD subgroup [adjusted HR, 3.021; 95% CI, 1.345-6.785; P=0.008]. Our findings suggest that miR-150 polymorphisms may contribute to the development of ischemic stroke and may potentially act as biomarkers to predict the risk of ischemic stroke. To the best of our knowledge, this is the first study to evaluate the association between miRNA polymorphisms (miR-34aC>A, miR-130aC>T, miR-150G>A and miR-155T>A) and ischemic stroke.

Introduction

Stroke is the second most common cause of mortality worldwide (1), and approximately 84% of these deaths are caused by ischemic stroke (2). In South Korea, stroke is the second most frequent cause of death after cancer and is more prevalent than heart disease. Stroke has well-known risk factors, including hypertension, diabetes mellitus, advanced age, smoking, hyperlipidemia, hyperhomocysteinemia and a thrombophilia event in blood vessels (1,2). A number of different diseases can cause ischemic stroke. The most common source of ischemic stroke is arterial occlusion in the head, usually caused by atherosclerosis, gradual cholesterol deposition or thrombosis (3,4). Blood vessel occlusion is primarily caused by thrombosis (53% of cases) or embolism (31% of cases) (5). Thrombus formation can be caused by excessive platelet generation (6), and the association between platelets and ischemic stroke has been widely studied (7-9). Platelets are involved in both normal hemostasis and thrombosis (10). They are formed in the cytoplasm of megakaryocytes (MKs), their precursor cells, located in the bone marrow (11).

MicroRNAs are a class of small (19-23 nt in length), endogenous non-coding RNA molecules that are endogenous physiological regulators of gene expression (12). miRNAs efficiently control gene expression by binding 3′-untranslated regions in mRNAs to downregulate their protein expression (13). miRNAs play important roles in a number of physiological and pathological processes, including metabolism (14), hematopoiesis (15) and immune function (16). Moreover, previous studies have indicated that temporal miRNA regulation may be related to the progression of ischemia in the cerebral artery (17,18). Furthermore, a recent study found a significant association between miRNA single nucleotide poly-
morphisms (SNPs) and the risk of ischemic stroke (19). As has been previously reported by Edelstein et al (20), as regards the regulated function of megakaryocytopoiesis, various miRNAs play a crucial role in this process, as well as in platelet biogenesis. Therefore, it may be valuable to investigate associations between MK formation-related miRNAs, such as miR-34a, miR-130a, miR-150 and miR-155 (20,21), and the prevalence of ischemic stroke.

In this study, we investigated the associations between 4 miRNA polymorphisms (miR-34a rs6577555C>A, miR-130a rs731384C>T, miR-150 rs73056059G>A and miR-155 rs767649T>A) and the risk of ischemic stroke in a Korean population.

Subjects and methods

Subjects. The study population comprised 596 patients with ischemic stroke (mean age ± SD: 63.67±10.42 years, 254 males, 342 females). Ischemic stroke was diagnosed based on rapidly developing neurological symptoms with a concurrent acute infarction, documented by brain magnetic resonance imaging (MRI). In addition, 404 control subjects were included (mean age ± SD: 63.66±10.47 years, 173 males, 231 females). The patients were enrolled by consecutive referral between July 1, 2000 and February 28, 2008 in the Neurology Department at CHA Bundang Medical Center (Seongnam, South Korea). The control subjects were recruited from patients who visited the hospital for a routine health examination. The exclusion criteria for the control subjects were as follows: a family history of stroke or experiencing non-specific dizziness, non-organic headaches, or anxiety during the enrollment period. All control subjects underwent some form of brain imaging (~75% MRI), and no organic cerebral lesions were observed. The subjects were interviewed to collect clinical information regarding demographic data and vascular risk factors. Subjects with a previous history of cerebral hemorrhage or those with incomplete medical histories were excluded from this study. The patients with ischemic stroke were classified into 3 subgroups as follows: 202 patients had left anterior descending artery (LAD) disease, 143 had small-vessel disease (SVD) and 57 had cardioembolism (CE); 194 patients had an undetermined etiology. Ischemic stroke was defined as a stroke (characterized by rapidly developing clinical symptoms and signs of focal and/or global brain function loss) with evidence of a cerebral infarction in clinically relevant areas of the brain based on a brain MRI. Based on clinical manifestations and neuroimaging data, two neurologists classified ischemic stroke into 3 etiological subtypes using the criteria from the Trial of Org 10172 in Acute Stroke Treatment (TOAST) clinical trial as follows (22): i) subtype 1: LAD, an infarct lesion of ≥15 mm in diameter, as determined by an MRI, and significant (>50%) stenosis of a major brain artery or a branch cortical artery, as determined by cerebral angiography, with symptoms associated with that arterial territory; ii) subtype 2: SVD, an infarct lesion of <15 mm, but >5 mm in diameter, as determined by an MRI, and classic lacunar syndrome without evidence of cerebral cortical dysfunction or a potentially detectable cardiac source for the embolism; and iii) subtype 3: CE, arterial occlusions presumably due to a heart-originated embolus, as detected by cardiac evaluation. We measured clinical parameters, including hypertension, diabetes, hyperlipidemia, homocysteine levels, folate levels, vitamin B12 levels, cholesterol, platelet (PLT) count, PT, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen levels, antithrombin levels, BUN levels and uric acid levels based on previously described methods (23,24).

Genetic analysis. Genomic DNA was extracted from blood leukocytes using the G-DEX blood extraction kit (Intron Inc., Seongnam, Korea). The 4 most well-studied SNPs in the miRNAs were determined by a documentary search which included promoter region SNPs (miR-34a rs6577555C>A, miR-130a rs731384C>T, miR-150 rs73056059G>A and miR-155 rs767649T>A). All SNP sequences were obtained from the HapMap database (www.hapmap.org) and dbSNP= (www.ncbi.nlm.nih.gov/projects/SNP). Nucleotide alterations were determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analyses using the isolated genomic DNA as a template. PCR for the miR-34a rs6577555C>A polymorphism was performed using the following primers: 5'-CCT GGT TAA CAT AGC CAG ACC-3' (forward) and 5'-GCA GAC ATG CTG ACT TTT CAA-3' (reverse). DNA was amplified over 35 cycles (denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec, and extension at 72°C for 35 sec). The PCR products were digested with the BanII restriction endonuclease (New England Biolabs, Beverly, MA, USA) at 37°C for 16 h and detected using 3% agarose gel electrophoresis. The primer sequences used to detect the miR-130a rs731384C>T polymorphism were 5'-GAT GCT CAG TCC TCA AAG AAC A-3' (forward) and 5'-TGA GTC GAG CTC TGG TTT AT-3' (reverse). DNA was amplified over 35 cycles (denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 35 sec). The PCR products were digested with NlaIII (New England Biolabs) at 37°C for 16 h and were visualized using 3% agarose gel electrophoresis. The primer sequences used to detect the miR-150 rs73056059G>A were 5'-GTT CCT GCC AGA AGG GTA G-3' (forward) and 5'-CTG GCC GCC CC-3' (reverse). DNA was amplified over 35 cycles (denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 40 sec). The products were digested with BccI (New England Biolabs) at 37°C for 16 h and detected using 4% gel electrophoresis. The primer sequences used to detect the miR-155 rs767649T>A polymorphism were 5'-CCT GTA TGA CAA GGT TGT GTT TG-3' (forward) and 5'-GCT GGC ATA CTA TTT CCA TAA-3' (reverse). DNA was amplified over 35 cycles (denaturation at 95°C for 35 sec, annealing at 56°C for 30 sec, and extension at 72°C for 35 sec). The PCR products were digested with Tsp45I (New England Biolabs) at 37°C for 16 h and visualized using 3% agarose gel electrophoresis.

Statistical analysis. Clinical characteristics were compared using the Student's unpaired t-test. Associations among ischemic stroke and the 4 miRNA genotypes were estimated by calculating the odd ratios (ORs) and 95% confidence intervals (CIs) using the Fisher's exact test. Adjusted ORs (AORs) for the miRNA polymorphisms were determined using multiple logistic regression analysis based on gender, age, diabetes mellitus, hypertension, hyperlipidemia and smoking. The genotype distribution for each polymorphism was assessed for Hardy-Weinberg equilib-
rimum deviations and genotype and allele frequency differences between groups were assessed using χ² tests. A value of P<0.05 was considered to indicate a statistically significant difference. Stratification analysis was used to distinguish stroke subgroups based on the size of the occluded vessel. One-way analysis of variance (ANOVA) was performed to compare the mean homocysteine concentration levels among different genotypes. Stats Direct Statistical Software (version 2.4.4; StatsDirect Ltd., Altrincham, UK) was used to calculate the adjusted OR and 95% CI. Survival curves were created using Cox proportional hazards regression. However, we could not find any polymorphism frequency differences between the ischemic stroke and control groups (Table II). Once the ischemic stroke group was stratified into subgroups and analyzed, we observed associations between miRNA polymorphisms and individual stroke subtypes. LAD was significantly associated with the miR-150A genotype (GG vs. AA: AOR, 1.570; 95% CI, 1.010-2.443), smokers (AOR, 1.651; 95% CI, 1.133-2.413), hypertension (AOR, 3.088; 95% CI, 2.089-4.566), hyperlipidemia (AOR, 6.060; 95% CI, 1.358-27.04) (these values are shown in bold in Table II). However, these differences dissipated once the P-value was adjusted for false discovery rate (FDR) (Table II). The miR-34aC>A, miR-130aC>T, and miR-155T>A polymorphisms did not differ significantly between the ischemic stroke and control subjects.

We performed stratified analyses according to age, gender, hypertension, diabetes mellitus, hyperlipidemia, smoking, folate levels and homocysteine levels. The miR-34aCA+AA genotype exhibited elevated prevalence in subjects who were ≥63 years of age (AOR, 1.443; 95% CI, 1.010-2.062), of the female gender (AOR, 1.459; 95% CI, 1.026-2.076) and who were non-diabetic (AOR, 1.360; 95% CI, 1.013-1.827). The miR-150A genotype also exhibited elevated prevalence in patients with hyperlipidemia (AOR, 6.060; 95% CI, 1.358-27.04) (Table III).

Combined gene-environment analyses revealed several genotypes that were associated with clinical factors related to the risk of ischemic stroke. The miR-34aCA+AA genotype exhibited elevated stroke prevalence in subjects with hypertension (AOR, 3.088; 95% CI, 2.089-4.566), hyperlipidemia (AOR, 1.570; 95% CI, 1.010-2.443), smokers (AOR, 1.651; 95% CI, 1.023-2.665) and those with high homocysteine levels.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (n=404)</th>
<th>Stroke patients (n=596)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>173 (42.8)</td>
<td>254 (42.6)</td>
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<tr>
<td>Age (years, mean ± SD)</td>
<td>63.66±10.47</td>
<td>63.67±10.42</td>
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<tr>
<td>Smoking (%)</td>
<td>138 (34.2)</td>
<td>211 (35.4)</td>
<td>0.704</td>
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<tr>
<td>Hypertension (%)</td>
<td>169 (41.8)</td>
<td>376 (63.1)</td>
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<tr>
<td>Diabetes mellitus (%)</td>
<td>54 (13.4)</td>
<td>161 (27.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
<td>95 (23.5)</td>
<td>179 (30.0)</td>
<td>0.070</td>
</tr>
<tr>
<td>Homocysteine (µmol/l, mean ± SD)</td>
<td>10.12±4.21</td>
<td>11.26±6.78</td>
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<td>Folate (nmol/l, mean ± SD)</td>
<td>8.88±7.99</td>
<td>7.06±5.29</td>
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<tr>
<td>Vitamin B₁₂ (pg/ml, mean ± SD)</td>
<td>746.18±670.06</td>
<td>747.46±624.35</td>
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<tr>
<td>Total cholesterol (mg/dl, mean ± SD)</td>
<td>193.70±37.59</td>
<td>189.88±40.79</td>
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<td>Triglyceride (mg/dl, mean ± SD)</td>
<td>147.74±90.39</td>
<td>152.58±114.39</td>
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<td>PLT (10⁹/ml, mean ± SD)</td>
<td>243.06±67.51</td>
<td>248.19±87.73</td>
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<td>PT (sec, mean ± SD)</td>
<td>11.77±0.79</td>
<td>11.91±3.08</td>
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<td>aPTT (sec, mean ± SD)</td>
<td>33.34±18.61</td>
<td>30.46±4.36</td>
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<td>Fibrinogen (mg/dl, mean ± SD)</td>
<td>397.83±119.62</td>
<td>426.93±132.40</td>
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<td>Antithrombin (% mean ± SD)</td>
<td>94.36±43.88</td>
<td>93.36±18.67</td>
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<td>BUN (mg/dl, mean ± SD)</td>
<td>15.93±5.02</td>
<td>16.29±7.80</td>
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<td>Uric acid (mg/dl, mean ± SD)</td>
<td>4.68±1.47</td>
<td>4.69±1.67</td>
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*P-values were calculated using a two-sided t-test for continuous variables and a Chi-square test for categorical variables. SD, standard deviation; PLT, platelet; PT, prothrombin time; aPTT, activated partial thromboplastin time; BUN, blood urea nitrogen.

Table I. Baseline characteristics between patients with ischemic stroke and the control subjects.
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (n=404)</th>
<th>Stroke patients (n=596)</th>
<th>AOR (95% CI)a</th>
<th>P-value</th>
<th>AOR (95% CI)a</th>
<th>P-value</th>
<th>AOR (95% CI)a</th>
<th>P-value</th>
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<tr>
<td>CC</td>
<td>239 (59.2)</td>
<td>323 (54.2)</td>
<td>1.000 (reference)</td>
<td></td>
<td>107 (53.0)</td>
<td>1.000 (reference)</td>
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<td>34 (59.6)</td>
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<td>CA</td>
<td>143 (35.4)</td>
<td>229 (38.4)</td>
<td>1.211 (0.915-1.603)</td>
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<td>82 (40.6)</td>
<td>1.326 (0.911-1.930)</td>
<td>0.140</td>
<td>58 (40.6)</td>
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<td>22 (5.4)</td>
<td>44 (7.4)</td>
<td>1.348 (0.787-2.431)</td>
<td>0.259</td>
<td>13 (6.4)</td>
<td>1.347 (0.622-2.919)</td>
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<td>10 (7.0)</td>
<td>1.244 (0.525-2.949)</td>
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<tr>
<td>Dominant</td>
<td>1.226 (0.938-1.602)</td>
<td>1.132 (0.927-1.905)</td>
<td>1.244 (0.525-2.949)</td>
<td>0.621</td>
<td>1.223 (0.494-2.552)</td>
<td>0.782</td>
<td>1.277 (0.412-3.952)</td>
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<tr>
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<td>1.252 (0.718-2.183)</td>
<td>0.969</td>
<td>0.975 (0.465-2.044)</td>
<td>0.947</td>
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<tr>
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<tr>
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<td>0.710 (0.420-1.200)</td>
<td>0.201</td>
<td>60.3 (28.6)</td>
<td>0.638 (0.360-1.131)</td>
<td>0.124</td>
</tr>
<tr>
<td>Dominant</td>
<td>1.024 (0.765-1.370)</td>
<td>0.876</td>
<td>1.006 (0.676-1.496)</td>
<td>0.977</td>
<td>0.786 (0.509-1.213)</td>
<td>0.276</td>
<td>0.552</td>
<td>1.280 (0.657-2.492)</td>
<td>0.468</td>
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</tr>
<tr>
<td>Recessive</td>
<td>0.728 (0.529-1.002)</td>
<td>0.156</td>
<td>0.676 (0.435-1.050)</td>
<td>0.082</td>
<td>0.246</td>
<td>0.651 (0.395-1.074)</td>
<td>0.093</td>
<td>0.230</td>
<td>0.659 (0.317-1.368)</td>
<td>0.263</td>
</tr>
</tbody>
</table>

*aAdjusted by age, gender, hypertension, diabetes mellitus, hyperlipidemia and smoking; †False discovery rate-adjusted P-value for multiple hypotheses testing using the Benjamini-Hochberg method. Values in bold font indicate statistical significance. AOR, adjusted odds ratio; 95% CI, 95% confidence interval; LAD, large-artery disease; SVD, small-vessel disease; CE, cardioembolism; N/A, not applicable.*
Table III. Effects of microRNA genotypes and characteristics of ischemic stroke among individual risk factors.

<table>
<thead>
<tr>
<th>Variables</th>
<th>miR-34a rs6577555CA+AA</th>
<th>P-value</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>miR-130a rs731384CT+TT</th>
<th>P-value</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>miR-150 rs7305605GA+AA</th>
<th>P-value</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>miR-155 rs767649TA</th>
<th>P-value</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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<tr>
<td>&lt;63</td>
<td>1.079 (0.720-1.617) 0.713 0.911</td>
<td>0.103 (0.608-1.746) 0.911 0.911</td>
<td>1.914 (0.848-4.318) 0.118 0.472</td>
<td>1.680 (1.037-2.722) 0.035 0.140</td>
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<tr>
<td>≥63</td>
<td>1.443 (1.010-2.062) 0.044 0.176</td>
<td>1.038 (0.673-1.601) 0.867 0.867</td>
<td>1.245 (0.628-2.469) 0.530 0.706</td>
<td>0.872 (0.576-1.319) 0.516 0.706</td>
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<tr>
<td>Gender</td>
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<tr>
<td>Male</td>
<td>1.016 (0.672-1.535) 0.941 0.941</td>
<td>0.978 (0.577-1.657) 0.934 0.941</td>
<td>1.628 (0.733-3.616) 0.232 0.464</td>
<td>1.026 (0.621-1.695) 0.921 0.921</td>
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<tr>
<td>Female</td>
<td>1.459 (1.026-2.076) 0.036 0.144</td>
<td>1.098 (0.708-1.703) 0.677 0.677</td>
<td>1.360 (0.679-2.725) 0.386 0.514</td>
<td>1.221 (0.814-1.830) 0.334 0.514</td>
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<tr>
<td>Hypertension</td>
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<tr>
<td>No</td>
<td>1.163 (0.793-1.705) 0.439 0.735</td>
<td>1.009 (0.618-1.649) 0.970 0.970</td>
<td>2.009 (0.939-4.297) 0.072 0.288</td>
<td>1.238 (0.798-1.922) 0.341 0.682</td>
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<tr>
<td>Yes</td>
<td>1.416 (0.972-2.063) 0.070 0.14</td>
<td>1.067 (0.672-1.695) 0.784 0.784</td>
<td>1.188 (0.591-2.387) 0.628 0.784</td>
<td>1.067 (0.680-1.674) 0.778 0.778</td>
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<td>Diabetes mellitus</td>
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<tr>
<td>No</td>
<td>1.360 (1.013-1.827) 0.041 0.164</td>
<td>1.028 (0.710-1.487) 0.885 0.885</td>
<td>1.514 (0.856-2.677) 0.154 0.229</td>
<td>1.044 (0.740-1.473) 0.806 0.920</td>
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<tr>
<td>Yes</td>
<td>0.962 (0.508-1.823) 0.906 0.906</td>
<td>1.103 (0.491-2.480) 0.812 0.906</td>
<td>1.566 (0.424-5.704) 0.505 0.906</td>
<td>1.761 (0.835-3.716) 0.137 0.548</td>
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<tr>
<td>No</td>
<td>1.328 (0.974-1.810) 0.073 0.264</td>
<td>1.017 (0.691-1.497) 0.933 0.933</td>
<td>1.057 (0.586-1.907) 0.853 0.933</td>
<td>1.096 (0.759-1.582) 0.625 0.998</td>
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<tr>
<td>Yes</td>
<td>1.100 (0.645-1.877) 0.726 0.828</td>
<td>1.078 (0.547-2.124) 0.828 0.828</td>
<td>6.060 (1.358-27.04) 0.018 0.072</td>
<td>1.379 (0.754-2.523) 0.297 0.594</td>
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<tr>
<td>No</td>
<td>1.160 (0.834-1.612) 0.378 0.645</td>
<td>1.163 (0.763-1.773) 0.484 0.645</td>
<td>1.143 (0.612-2.137) 0.675 0.675</td>
<td>1.300 (0.886-1.906) 0.180 0.720</td>
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<tr>
<td>Yes</td>
<td>1.460 (0.923-2.308) 0.105 0.144</td>
<td>0.890 (0.508-1.557) 0.682 0.682</td>
<td>2.604 (0.936-7.241) 0.067 0.144</td>
<td>0.846 (0.485-1.473) 0.554 0.554</td>
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<td>Folate&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>&gt;3.56 nmol/l</td>
<td>1.228 (0.919-1.641) 0.165 0.330</td>
<td>1.071 (0.749-1.531) 0.707 0.707</td>
<td>1.319 (0.757-2.299) 0.328 0.437</td>
<td>1.190 (0.847-1.672) 0.317 0.437</td>
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<tr>
<td>≤3.56 nmol/l</td>
<td>0.960 (0.411-2.244) 0.925 0.925</td>
<td>1.795 (0.466-6.917) 0.395 0.592</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.984 (0.360-2.689) 0.974 0.974</td>
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<td>Homocysteine&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>&lt;13.92 µmol/l</td>
<td>1.184 (0.889-1.579) 0.249 0.332</td>
<td>0.994 (0.698-1.415) 0.973 0.973</td>
<td>1.422 (0.808-2.502) 0.223 0.332</td>
<td>1.180 (0.846-1.647) 0.331 0.621</td>
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<tr>
<td>≥13.92 µmol/l</td>
<td>1.858 (0.868-3.975) 0.110 0.44</td>
<td>2.068 (0.614-6.969) 0.241 0.482</td>
<td>1.929 (0.467-7.966) 0.364 0.485</td>
<td>0.959 (0.362-2.545) 0.933 0.933</td>
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</tbody>
</table>

*The adjusted odds ratio is the ratio of the odds of ischemic stroke among individuals with microRNA genotypes to the odds of ischemic stroke among individuals without microRNA genotypes, adjusted for age, gender, hypertension, diabetes mellitus, hyperlipidemia, smoking, folate, and homocysteine. The adjusted odds ratio is further adjusted for multiple hypotheses testing using the Benjamini-Hochberg method.*

<sup>a</sup>Folate 3.56 nmol/l was the lower 15% cut-off folate level in ischemic stroke patients and controls; homocysteine 13.92 µmol/l was the upper 15% cut-off homocysteine level in ischemic stroke patients and controls. Values in bold font indicate statistical significance. AOR, adjusted odds ratio; 95% CI, 95% confidence interval.
Table IV. Ischemic stroke incidence by interactions with environmental factors such as gender, advanced age, hypertension, diabetes mellitus, hyperlipidemia, smoking, folate levels and homocysteine levels.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>miR-34a rs6577555CC</th>
<th>miR-34a rs6577555CA+AA</th>
<th>miR-130a rs731384CC</th>
<th>miR-130a rs731384CT+TT</th>
<th>miR-150 rs7305605GG</th>
<th>miR-150 rs7305605GA+AA</th>
<th>miR-155 rs767649TT</th>
<th>miR-155 rs767649TA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1.000 (reference)</td>
<td>1.010 (0.668-1.527)</td>
<td>1.000 (reference)</td>
<td>0.978 (0.578-1.656)</td>
<td>1.000 (reference)</td>
<td>1.614 (0.725-3.589)</td>
<td>1.000 (reference)</td>
<td>1.026 (0.621-1.695)</td>
</tr>
<tr>
<td>Female</td>
<td>0.800 (0.523-1.225)</td>
<td>1.437 (0.897-2.301)</td>
<td>1.067 (0.745-1.530)</td>
<td>1.169 (0.699-1.953)</td>
<td>1.034 (0.744-1.437)</td>
<td>1.737 (0.804-3.753)</td>
<td>0.895 (0.494-1.622)</td>
<td>1.083 (0.619-1.893)</td>
</tr>
<tr>
<td>Age &lt;65</td>
<td>1.000 (reference)</td>
<td>1.079 (0.720-1.617)</td>
<td>1.000 (reference)</td>
<td>1.030 (0.608-1.746)</td>
<td>1.000 (reference)</td>
<td>1.914 (0.848-4.318)</td>
<td>1.000 (reference)</td>
<td>1.680 (1.037-2.722)</td>
</tr>
<tr>
<td>Age ≥65</td>
<td>0.790 (0.546-1.145)</td>
<td>1.158 (0.779-1.723)</td>
<td>0.864 (0.635-1.176)</td>
<td>0.882 (0.555-1.404)</td>
<td>0.876 (0.658-1.167)</td>
<td>1.184 (0.584-2.398)</td>
<td>1.404 (0.828-2.379)</td>
<td>1.098 (0.672-1.795)</td>
</tr>
<tr>
<td>Without hypertension</td>
<td>1.000 (reference)</td>
<td>1.154 (0.787-1.694)</td>
<td>1.000 (reference)</td>
<td>1.005 (0.616-1.639)</td>
<td>1.000 (reference)</td>
<td>2.013 (0.939-4.317)</td>
<td>1.000 (reference)</td>
<td>1.238 (0.798-1.922)</td>
</tr>
<tr>
<td>With hypertension</td>
<td>2.157 (1.517-3.069)</td>
<td>3.088 (2.089-4.566)</td>
<td>2.300 (1.711-3.093)</td>
<td>2.390 (1.483-3.850)</td>
<td>2.385 (1.808-3.146)</td>
<td>2.871 (1.416-5.824)</td>
<td>2.697 (1.612-4.511)</td>
<td>2.756 (1.773-4.285)</td>
</tr>
<tr>
<td>Without diabetes mellitus</td>
<td>1.000 (reference)</td>
<td>1.357 (1.011-1.823)</td>
<td>1.000 (reference)</td>
<td>1.023 (0.707-1.479)</td>
<td>1.000 (reference)</td>
<td>1.513 (0.856-2.675)</td>
<td>1.000 (reference)</td>
<td>1.044 (0.740-1.473)</td>
</tr>
<tr>
<td>With diabetes mellitus</td>
<td>2.452 (1.550-3.879)</td>
<td>2.352 (1.398-3.957)</td>
<td>2.096 (1.423-3.088)</td>
<td>2.205 (1.043-4.661)</td>
<td>2.115 (1.476-3.032)</td>
<td>3.161 (0.883-11.31)</td>
<td>1.612 (0.852-3.050)</td>
<td>2.945 (1.659-5.228)</td>
</tr>
<tr>
<td>Without hyperlipidemia</td>
<td>1.000 (reference)</td>
<td>1.323 (0.970-1.804)</td>
<td>1.000 (reference)</td>
<td>1.017 (0.691-1.497)</td>
<td>1.000 (reference)</td>
<td>1.057 (0.886-1.908)</td>
<td>1.000 (reference)</td>
<td>1.096 (0.759-1.582)</td>
</tr>
<tr>
<td>With hyperlipidemia</td>
<td>1.463 (0.974-2.196)</td>
<td>1.570 (1.010-2.443)</td>
<td>1.324 (0.945-1.857)</td>
<td>1.409 (0.754-2.634)</td>
<td>1.214 (0.887-1.662)</td>
<td>7.215 (1.665-31.345)</td>
<td>1.377 (0.795-2.387)</td>
<td>1.734 (1.049-2.865)</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>1.000 (reference)</td>
<td>1.158 (0.833-1.610)</td>
<td>1.000 (reference)</td>
<td>1.159 (0.761-1.767)</td>
<td>1.000 (reference)</td>
<td>1.140 (0.610-2.130)</td>
<td>1.000 (reference)</td>
<td>1.300 (0.886-1.906)</td>
</tr>
<tr>
<td>Smoker</td>
<td>0.868 (0.566-1.330)</td>
<td>1.651 (1.023-2.665)</td>
<td>1.215 (0.843-1.750)</td>
<td>1.132 (0.650-1.971)</td>
<td>1.066 (0.765-1.486)</td>
<td>3.594 (1.267-10.20)</td>
<td>1.570 (0.848-2.907)</td>
<td>1.530 (0.770-2.205)</td>
</tr>
<tr>
<td>Folate &gt;3.56 nmol/l*</td>
<td>1.000 (reference)</td>
<td>1.227 (0.918-1.640)</td>
<td>1.000 (reference)</td>
<td>1.071 (0.750-1.531)</td>
<td>1.000 (reference)</td>
<td>1.322 (0.758-2.304)</td>
<td>1.000 (reference)</td>
<td>1.190 (0.847-1.672)</td>
</tr>
<tr>
<td>Homocysteine &lt;13.92 µmol/l</td>
<td>1.000 (reference)</td>
<td>1.182 (0.887-1.575)</td>
<td>1.000 (reference)</td>
<td>0.996 (0.699-1.418)</td>
<td>1.000 (reference)</td>
<td>1.415 (0.803-2.493)</td>
<td>1.000 (reference)</td>
<td>1.180 (0.846-1.647)</td>
</tr>
<tr>
<td>Homocysteine ≥13.92 µmol/l</td>
<td>1.308 (0.763-2.243)</td>
<td>2.230 (1.236-4.024)</td>
<td>1.437 (0.936-2.207)</td>
<td>2.788 (0.891-8.718)</td>
<td>1.504 (0.994-2.274)</td>
<td>3.004 (0.804-11.23)</td>
<td>1.627 (0.697-3.797)</td>
<td>1.327 (0.742-2.372)</td>
</tr>
</tbody>
</table>

*Folate 3.56 nmol/l was the lower 15% cut-off folate level in ischemic stroke patients and controls; †homocysteine 13.92 µmol/l was the upper 15% cut-off homocysteine level in ischemic stroke patients and controls. Values in bold font indicate statistical significance.
The miR-130a CT+TT genotype also exhibited elevated stroke prevalence in subjects with hypertension (AOR, 2.390; 95% CI, 1.483-3.850), diabetes mellitus (AOR, 2.205; 95% CI, 1.043-4.661) and low folate levels (AOR, 4.702; 95% CI, 1.341-16.49). The miR-150 GA+AA genotype exhibited elevated stroke prevalence in subjects with hypertension (AOR, 2.871; 95% CI, 1.416-5.824), hyperlipidemia (AOR, 7.215; 95% CI, 1.655-31.45) and who were smokers (AOR, 3.594; 95% CI, 1.041-10.20). The miR-155 TA genotype exhibited elevated stroke prevalence in subjects with diabetes mellitus (AOR, 2.945; 95% CI, 1.659-5.228) and hyperlipidemia (AOR, 1.734; 95% CI, 1.049-2.865) (Table IV).

We then performed allelic combination analyses using the multifactor dimensionality reduction method comparing the ischemic stroke patients and the control subjects (Table V). The following allele combinations exhibited a significant association with the prevalence of stroke (P>0.05): the A-T-G-A allele combination of miR-34a C>A/miR-130a C>T/miR-150 G>A/miR-155 T>A (OR, 0.052; 95% CI, 0.003-0.921), the A-C-A allele combination of miR-34a C>A/miR-130a C>T/miR-150 G>A (OR, 4.285; 95% CI, 1.255-14.62), the A-A allele combination of miR-34a C>A/miR-150 G>A (OR, 3.814; 95% CI, 1.106-13.15), and the A-A allele combination of miR-150 G>A/miR-155 T>A (OR, 1.970; 95% CI, 1.013-3.831). We also performed genotype combination analyses. The

Table V. Allele combination analysis for the microRNA polymorphisms in patients with ischemic stroke and controls by MDR.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overall (2n=2000)</th>
<th>Controls (2n=808)</th>
<th>Stroke patients (2n=1192)</th>
<th>OR (95% CI)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-34a C&gt;A/miR-130a C&gt;T/miR-150 G&gt;A/miR-155 T&gt;A</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>C-C-G-T</td>
<td>0.341</td>
<td>0.341</td>
<td>0.344</td>
<td>1.000</td>
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</tr>
<tr>
<td>C-C-G-A</td>
<td>0.308</td>
<td>0.330</td>
<td>0.292</td>
<td>0.878</td>
<td>0.260</td>
</tr>
<tr>
<td>C-C-A-T</td>
<td>0.013</td>
<td>0.014</td>
<td>0.008</td>
<td>0.610</td>
<td>0.268</td>
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<tr>
<td>C-C-A-A</td>
<td>0.012</td>
<td>0.011</td>
<td>0.015</td>
<td>1.341</td>
<td>0.552</td>
</tr>
<tr>
<td>C-T-G-T</td>
<td>0.037</td>
<td>0.042</td>
<td>0.033</td>
<td>0.789</td>
<td>0.384</td>
</tr>
<tr>
<td>C-T-G-A</td>
<td>0.033</td>
<td>0.030</td>
<td>0.036</td>
<td>1.202</td>
<td>0.516</td>
</tr>
<tr>
<td>C-T-A-T</td>
<td>0.003</td>
<td>0.001</td>
<td>0.004</td>
<td>3.354</td>
<td>0.410</td>
</tr>
<tr>
<td>C-T-A-A</td>
<td>0.001</td>
<td>0.000</td>
<td>0.002</td>
<td>3.356</td>
<td>0.519</td>
</tr>
<tr>
<td>A-C-G-T</td>
<td>0.119</td>
<td>0.113</td>
<td>0.119</td>
<td>1.047</td>
<td>0.816</td>
</tr>
<tr>
<td>A-C-G-A</td>
<td>0.098</td>
<td>0.092</td>
<td>0.104</td>
<td>1.124</td>
<td>0.510</td>
</tr>
<tr>
<td>A-C-A-T</td>
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<td>0.000</td>
<td>0.005</td>
<td>8.725</td>
<td>0.086</td>
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<tr>
<td>A-C-A-A</td>
<td>0.008</td>
<td>0.004</td>
<td>0.009</td>
<td>2.236</td>
<td>0.263</td>
</tr>
<tr>
<td>A-T-G-T</td>
<td>0.022</td>
<td>0.016</td>
<td>0.028</td>
<td>1.703</td>
<td>0.121</td>
</tr>
<tr>
<td>A-T-G-A</td>
<td>0.005</td>
<td>0.008</td>
<td>0.000</td>
<td>0.052</td>
<td>0.004</td>
</tr>
<tr>
<td>A-T-A-A</td>
<td>0.000</td>
<td>0.000</td>
<td>0.002</td>
<td>3.356</td>
<td>0.519</td>
</tr>
<tr>
<td>miR-34a C&gt;A/miR-130a C&gt;T/miR-150 G&gt;A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-C-G</td>
<td>0.650</td>
<td>0.670</td>
<td>0.637</td>
<td>1.000</td>
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<tr>
<td>C-C-A</td>
<td>0.024</td>
<td>0.025</td>
<td>0.023</td>
<td>0.964</td>
<td>1.000</td>
</tr>
<tr>
<td>C-T-G</td>
<td>0.070</td>
<td>0.072</td>
<td>0.069</td>
<td>1.010</td>
<td>1.000</td>
</tr>
<tr>
<td>C-T-A</td>
<td>0.004</td>
<td>0.001</td>
<td>0.006</td>
<td>4.999</td>
<td>0.150</td>
</tr>
<tr>
<td>A-C-G</td>
<td>0.216</td>
<td>0.205</td>
<td>0.222</td>
<td>1.143</td>
<td>0.258</td>
</tr>
<tr>
<td>A-C-A</td>
<td>0.010</td>
<td>0.004</td>
<td>0.015</td>
<td>4.285</td>
<td>0.013</td>
</tr>
<tr>
<td>A-T-G</td>
<td>0.027</td>
<td>0.023</td>
<td>0.029</td>
<td>1.315</td>
<td>0.399</td>
</tr>
<tr>
<td>miR-34a C&gt;A/miR-150 G&gt;A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-G</td>
<td>0.719</td>
<td>0.743</td>
<td>0.704</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>C-A</td>
<td>0.029</td>
<td>0.026</td>
<td>0.030</td>
<td>1.226</td>
<td>0.496</td>
</tr>
<tr>
<td>A-G</td>
<td>0.243</td>
<td>0.228</td>
<td>0.252</td>
<td>1.170</td>
<td>0.149</td>
</tr>
<tr>
<td>A-A</td>
<td>0.009</td>
<td>0.004</td>
<td>0.014</td>
<td>3.814</td>
<td>0.032</td>
</tr>
<tr>
<td>miR-150 G&gt;A/miR-155 T&gt;A</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-T</td>
<td>0.520</td>
<td>0.512</td>
<td>0.528</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>G-A</td>
<td>0.443</td>
<td>0.459</td>
<td>0.429</td>
<td>0.904</td>
<td>0.285</td>
</tr>
<tr>
<td>A-T</td>
<td>0.016</td>
<td>0.014</td>
<td>0.014</td>
<td>0.876</td>
<td>0.845</td>
</tr>
<tr>
<td>A-A</td>
<td>0.023</td>
<td>0.015</td>
<td>0.030</td>
<td>1.970</td>
<td>0.049</td>
</tr>
</tbody>
</table>

95% CI, 95% confidence interval; OR, odd ratios; *Fisher's exact test. MDR, multifactor dimensionality reduction. Values in bold font indicate statistical significance.
Table VI. Combined genotype analysis for the microRNA polymorphisms in patients with ischemic stroke and controls.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (n=404)</th>
<th>Stroke patients (n=596)</th>
<th>AOR (95% CI)(^a)</th>
<th>P-value(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>miR-34a&gt;C&gt;A/miR-130a&gt;C&gt;T</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC-CC</td>
<td>193 (47.8)</td>
<td>260 (43.6)</td>
<td>1.000 (reference)</td>
<td></td>
</tr>
<tr>
<td>CC-CT</td>
<td>45 (11.1)</td>
<td>57 (9.6)</td>
<td>0.946 (0.601-1.488)</td>
<td>0.809</td>
</tr>
<tr>
<td>CC-TT</td>
<td>1 (0.2)</td>
<td>6 (1.0)</td>
<td>4.979 (0.566-43.81)</td>
<td>0.148</td>
</tr>
<tr>
<td>CA-CC</td>
<td>119 (29.5)</td>
<td>187 (31.4)</td>
<td>1.231 (0.904-1.675)</td>
<td>0.187</td>
</tr>
<tr>
<td>CA-CT</td>
<td>23 (5.7)</td>
<td>41 (6.9)</td>
<td>1.245 (0.700-2.214)</td>
<td>0.456</td>
</tr>
<tr>
<td>CA-TT</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
<td>0.816 (0.050-13.29)</td>
<td>0.887</td>
</tr>
<tr>
<td>AA-CC</td>
<td>16 (4.0)</td>
<td>32 (5.4)</td>
<td>1.405 (0.721-2.740)</td>
<td>0.318</td>
</tr>
<tr>
<td>AA-CT</td>
<td>6 (1.5)</td>
<td>12 (2.0)</td>
<td>2.201 (0.752-6.445)</td>
<td>0.150</td>
</tr>
<tr>
<td><strong>miR-34a&gt;C&gt;A/miR-150G&gt;A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC-GG</td>
<td>220 (54.5)</td>
<td>297 (49.8)</td>
<td>1.000 (reference)</td>
<td></td>
</tr>
<tr>
<td>CC-GA</td>
<td>19 (4.7)</td>
<td>26 (4.4)</td>
<td>1.036 (0.543-1.977)</td>
<td>0.915</td>
</tr>
<tr>
<td>CA-GG</td>
<td>140 (34.7)</td>
<td>207 (34.7)</td>
<td>1.134 (0.851-1.513)</td>
<td>0.391</td>
</tr>
<tr>
<td>CA-GA</td>
<td>3 (0.7)</td>
<td>22 (3.7)</td>
<td>5.470 (1.580-18.93)</td>
<td>0.007</td>
</tr>
<tr>
<td>AA-GG</td>
<td>20 (5.0)</td>
<td>40 (6.7)</td>
<td>1.570 (0.869-2.836)</td>
<td>0.135</td>
</tr>
<tr>
<td>AA-GA</td>
<td>2 (0.5)</td>
<td>4 (0.7)</td>
<td>1.329 (0.220-8.028)</td>
<td>0.757</td>
</tr>
<tr>
<td><strong>miR-34a&gt;C&gt;A/miR-155T&gt;A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC-TT</td>
<td>64 (15.8)</td>
<td>90 (15.1)</td>
<td>1.000 (reference)</td>
<td></td>
</tr>
<tr>
<td>CC-TA</td>
<td>115 (28.5)</td>
<td>166 (27.9)</td>
<td>1.081 (0.706-1.653)</td>
<td>0.721</td>
</tr>
<tr>
<td>CC-AA</td>
<td>60 (14.9)</td>
<td>67 (11.2)</td>
<td>0.834 (0.508-1.367)</td>
<td>0.471</td>
</tr>
<tr>
<td>CA-TT</td>
<td>49 (12.1)</td>
<td>63 (10.6)</td>
<td>1.055 (0.624-1.578)</td>
<td>0.841</td>
</tr>
<tr>
<td>CA-TA</td>
<td>65 (16.1)</td>
<td>120 (20.1)</td>
<td>1.428 (0.891-2.287)</td>
<td>0.139</td>
</tr>
<tr>
<td>CA-AA</td>
<td>29 (7.2)</td>
<td>46 (7.7)</td>
<td>1.020 (0.561-1.855)</td>
<td>0.949</td>
</tr>
<tr>
<td>AA-TT</td>
<td>4 (1.0)</td>
<td>14 (2.3)</td>
<td>3.351 (0.965-11.64)</td>
<td>0.057</td>
</tr>
<tr>
<td>AA-TA</td>
<td>11 (2.7)</td>
<td>25 (4.2)</td>
<td>1.892 (0.820-4.366)</td>
<td>0.135</td>
</tr>
<tr>
<td>AA-AA</td>
<td>7 (1.7)</td>
<td>5 (0.8)</td>
<td>0.347 (0.091-1.317)</td>
<td>0.120</td>
</tr>
<tr>
<td><strong>miR-130a&gt;C&gt;T/miR-150G&gt;A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC-GG</td>
<td>307 (76.0)</td>
<td>438 (73.5)</td>
<td>1.000 (reference)</td>
<td></td>
</tr>
<tr>
<td>CC-GA</td>
<td>21 (5.2)</td>
<td>41 (6.9)</td>
<td>1.308 (0.742-2.306)</td>
<td>0.354</td>
</tr>
<tr>
<td>CT-GG</td>
<td>71 (17.6)</td>
<td>101 (16.9)</td>
<td>0.965 (0.679-1.371)</td>
<td>0.841</td>
</tr>
<tr>
<td>CT-GA</td>
<td>3 (0.7)</td>
<td>9 (1.5)</td>
<td>2.292 (0.590-8.897)</td>
<td>0.231</td>
</tr>
<tr>
<td>TT-GG</td>
<td>2 (0.5)</td>
<td>5 (0.8)</td>
<td>1.660 (0.311-8.879)</td>
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</tr>
<tr>
<td><strong>miR-130a&gt;C&gt;T/miR-155T&gt;A</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC-TT</td>
<td>91 (22.5)</td>
<td>135 (22.7)</td>
<td>1.000 (reference)</td>
<td></td>
</tr>
<tr>
<td>CC-TA</td>
<td>156 (38.6)</td>
<td>243 (40.8)</td>
<td>1.057 (0.745-1.499)</td>
<td>0.757</td>
</tr>
<tr>
<td>CC-AA</td>
<td>81 (20.0)</td>
<td>101 (16.9)</td>
<td>0.747 (0.493-1.131)</td>
<td>0.168</td>
</tr>
<tr>
<td>CT-TT</td>
<td>26 (6.4)</td>
<td>29 (4.9)</td>
<td>0.718 (0.384-1.344)</td>
<td>0.301</td>
</tr>
<tr>
<td>CT-TA</td>
<td>33 (8.2)</td>
<td>64 (10.7)</td>
<td>1.217 (0.718-2.062)</td>
<td>0.465</td>
</tr>
<tr>
<td>CT-AA</td>
<td>15 (3.7)</td>
<td>17 (2.9)</td>
<td>0.747 (0.345-1.618)</td>
<td>0.459</td>
</tr>
<tr>
<td>TT-TA</td>
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<td>4 (0.7)</td>
<td>1.395 (0.245-7.949)</td>
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<tr>
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<tr>
<td>GG-TT</td>
<td>109 (27.0)</td>
<td>161 (27.0)</td>
<td>1.000 (reference)</td>
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<tr>
<td>GG-TA</td>
<td>183 (45.3)</td>
<td>273 (45.8)</td>
<td>1.008 (0.730-1.392)</td>
<td>0.962</td>
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<tr>
<td>GG-AA</td>
<td>88 (21.8)</td>
<td>110 (18.5)</td>
<td>0.796 (0.540-1.173)</td>
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</tr>
<tr>
<td>GA-TT</td>
<td>8 (2.0)</td>
<td>6 (1.0)</td>
<td>0.570 (0.181-1.799)</td>
<td>0.338</td>
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<tr>
<td>GA-TA</td>
<td>8 (2.0)</td>
<td>38 (6.4)</td>
<td>3.265 (1.426-7.474)</td>
<td>0.005</td>
</tr>
<tr>
<td>GA-AA</td>
<td>8 (2.0)</td>
<td>8 (1.3)</td>
<td>0.519 (0.176-1.534)</td>
<td>0.236</td>
</tr>
</tbody>
</table>

\(^a\)Adjusted by age, gender, hypertension, diabetes mellitus, hyperlipidemia and smoking; \(^b\)ORs and 95% CIs of each specific genotype were calculated with reference to frequencies of all others. Values in bold font indicate statistical significance. CI, confidence interval; OR, odd ratios.
Table VII. Clinical variables in patients with ischemic stroke, stratified by the microRNA polymorphisms status.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Vitamin B12 (pg/ml)</th>
<th>Fibrinogen (mg/dl)</th>
<th>Antithrombin(%)</th>
<th>aPTT (sec)</th>
<th>PT (sec)</th>
<th>PLT(10^3/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>P-value (^{a})</td>
<td>Mean ± SD</td>
<td>P-value (^{a})</td>
<td>Mean ± SD</td>
<td>P-value (^{a})</td>
</tr>
<tr>
<td>miR-34a rs6577555C&gt;A</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>CC</td>
<td>768.51±716.83</td>
<td>0.990</td>
<td>424.43±132.54</td>
<td>0.850</td>
<td>92.85±18.53</td>
<td>0.836(^{b})</td>
</tr>
<tr>
<td>CA</td>
<td>713.18±498.85</td>
<td></td>
<td>416.72±129.65</td>
<td></td>
<td>92.44±17.86</td>
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</tr>
<tr>
<td>AA</td>
<td>752.24±692.82</td>
<td></td>
<td>415.26±117.21</td>
<td></td>
<td>105.02±70.81</td>
<td></td>
</tr>
<tr>
<td>Dominant (CC vs. CA+AA)</td>
<td>719.16±532.37</td>
<td>0.226(^{c})</td>
<td>416.49±127.54</td>
<td>0.430</td>
<td>94.46±32.87</td>
<td>0.770(^{c})</td>
</tr>
<tr>
<td>Recessive (CC+CA vs. AA)</td>
<td>752.24±692.82</td>
<td>0.945</td>
<td>415.26±117.21</td>
<td>0.754</td>
<td>105.02±70.81</td>
<td>0.557(^{c})</td>
</tr>
<tr>
<td>miR-130a rs731384C&gt;T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>749.11±674.55</td>
<td>0.738</td>
<td>411.65±124.83</td>
<td>&lt;0.001</td>
<td>93.69±27.86</td>
<td>0.860</td>
</tr>
<tr>
<td>CT</td>
<td>746.52±502.57</td>
<td></td>
<td>462.34±147.50</td>
<td></td>
<td>93.30±15.50</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>563.89±167.02</td>
<td></td>
<td>429.80±94.44</td>
<td>88.00±10.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant (CC vs. CT+TT)</td>
<td>738.01±493.35</td>
<td>0.848(^{c})</td>
<td>461.05±145.66</td>
<td>0.0009(^{c})</td>
<td>93.06±15.31</td>
<td>0.952(^{c})</td>
</tr>
<tr>
<td>Recessive (CC+CT vs. TT)</td>
<td>563.89±167.02</td>
<td>0.207(^{c})</td>
<td>429.80±94.44</td>
<td>0.878</td>
<td>88.00±10.56</td>
<td>0.389(^{c})</td>
</tr>
<tr>
<td>miR-150 rs73056059G&gt;A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>748.05±660.94</td>
<td>0.738</td>
<td>418.79±129.21</td>
<td>0.116</td>
<td>93.59±26.44</td>
<td>0.948</td>
</tr>
<tr>
<td>GA</td>
<td>733.68±365.56</td>
<td></td>
<td>444.18±141.02</td>
<td></td>
<td>93.36±19.96</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>N/A</td>
<td></td>
<td>N/A</td>
<td></td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Dominant (GG vs. GA+AA)</td>
<td>733.68±365.56</td>
<td>0.298(^{c})</td>
<td>444.18±141.02</td>
<td>0.163</td>
<td>93.36±19.96</td>
<td>0.808(^{c})</td>
</tr>
<tr>
<td>Recessive (GG+GA vs. AA)</td>
<td>N/A</td>
<td></td>
<td>N/A</td>
<td></td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>miR-155 rs767649T&gt;A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>725.86±550.21</td>
<td>0.287</td>
<td>415.63±129.13</td>
<td>0.055</td>
<td>97.42±39.69</td>
<td>0.049</td>
</tr>
<tr>
<td>TA</td>
<td>772.02±713.53</td>
<td></td>
<td>432.23±134.92</td>
<td></td>
<td>91.92±17.99</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>716.23±580.51</td>
<td></td>
<td>400.34±117.63</td>
<td></td>
<td>92.29±16.25</td>
<td></td>
</tr>
<tr>
<td>Dominant (TT vs. TA+AA)</td>
<td>755.38±676.66</td>
<td>0.620(^{c})</td>
<td>423.01±130.84</td>
<td>0.505</td>
<td>92.03±17.47</td>
<td>0.157(^{c})</td>
</tr>
<tr>
<td>Recessive (TT+TA vs. AA)</td>
<td>716.23±580.51</td>
<td>0.030(^{c})</td>
<td>400.34±117.63</td>
<td>0.036</td>
<td>92.29±16.25</td>
<td>0.644(^{c})</td>
</tr>
</tbody>
</table>

\(^{a}\)Calculated using ANOVA; \(^{b}\)calculated using the Kruskal-Wallis test; \(^{c}\)calculated using the Mann-Whitney test. Values in bold indicate statistical significance. PLT, platelet; PT, prothrombin time; aPTT, activated partial thromboplastin time; SD, standard deviation.
miR-34a and miR-150 polymorphisms were associated with increased prevalence of ischemic stroke (Table VI). The miR-130a genotype was associated with fibrinogen levels (CC vs. CT vs. TT: CC, 411.65±124.83; CT, 462.34±147.50; TT, 429.80±94.44; P<0.001). The miR-150 dominant model (GG vs. GA+AA) was significantly associated with increased platelet counts (GG vs. GA+AA: GG, 244.44±66.30; GA+AA, 266.53±58.58; P=0.002). The miR-155 recessive model (TT+TA vs. AA) was associated with vitamin B12 (TT+TA vs. AA: TT+TA, 748.94±631.87; AA, 716.23±580.51; P=0.030) and fibrinogen levels (TT+TA vs. AA: TT+TA, 423.93±177.56; AA, 400.34±117.63; P=0.036), and the miR-155 genotype was associated with antithrombin levels (TT vs. TA vs. AA: TT, 97.42±39.69; TA, 91.92±79.99; AA, 92.29±16.25; P=0.049) (Table VII).

The association between the miRNA polymorphisms and ischemic stroke patient survival is shown (Figs. 1 and 2). Cox proportional analysis indicated that the miR-34a CA genotype was significantly associated with survival in the SVD subgroup (CC vs. CA, P=0.016) (Fig. 1A), (CC vs. CA+AA, P=0.019) (Fig. 1B). The miR-150GA genotype was associated with survival in the LAD subgroup (GG vs. GA, P=0.009) (Fig. 2). We then investigated the promoter binding site for the miR-150 polymorphism.

**Discussion**

In this study, we selected a few potentially relevant miRNA SNPs, miR-34a rs6577555C>A, miR-130a rs731384C>T, miR-150 rs73056059G>A and miR-155 rs767649T>A, and investigated their association with ischemic stroke in a Korean population. We found that the miR-150G>A polymorphism may be associated with a susceptibility for LAD and CE ischemic stroke. In the gene-environment interaction analysis, the miR-150GA+AA genotype combined with hyperlipidemia or smoking exhibited a significantly higher prevalence of ischemic stroke. In the allelic combination analysis, the A-A allele (miR-34a C>A and miR-150 G>A) significantly increased ischemic stroke prevalence overall. In particular, subjects with the miR-150A allele exhibited a marked increase in ischemic stroke incidence, whereas subjects with the miR-150G allele had a decreased prevalence of ischemic stroke. These data were confirmed in combination analysis, where the miR-150GA+AA genotype also exhibited an increased prevalence of ischemic stroke. The miR-150GA genotype was also associated with a high adjusted HR in the survival analysis in both the overall ischemic stroke patients and the LAD and CE subtypes. Additionally, subjects with the miR-150GA genotype had significantly higher platelet counts.

miRNAs play crucial roles in a number of physiological and pathological processes, including neurodegenerative diseases (25), epigenetics (26,27), coronary artery disease (28), metabolism (14), tumorogenesis (29,30), angiogenesis and colonization (31). miRNAs play roles in oncogenesis, heart disease and nervous system function. It has been suggested that miRNAs may be used as biomarkers for cardiovascular diseases, including coronary artery disease, stroke, acute myocardial infarction and heart failure (17,32-34).
miR-34a regulates the myeloblastosis transcription factor MYB (35); miR-130a regulates V-maf avian musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB) (36); miR-150 regulates c-MYB (37); and miR-155 regulates V-Ets avian erythroblastosis virus E26 oncogene homolog-1 (ETS-1) and Meis homeobox-1 (MEIS-1) (38). Previous studies have reported that these genes contribute to suppressing megakaryocyte-megakaryocyte and megakaryocyte differentiation (36,39,40). MKs are platelet precursors, and excessive MK differentiation results in abnormal platelet production. Studies have been carried out investigating the causal association between increased platelets and ischemic stroke risk (41-43).

We then searched for transcription factors that could potentially bind the miR-150 promoter near the rs73056059G>A nucleotide. For the A allele, we found an additional transcription binding site for LyF-1 and YY-1, which could alter miR-150 levels (44). Transcription factor YY1 is associated with increased promoter activity (45-47); therefore, we hypothesize that miR-150 expression is increased by YY1 in subjects with an A allele. Supporting this hypothesis, we performed an ANOVA, which indicated that the miR-150GA genotype had higher platelet counts than the GG type. However, we were unable to directly measure promoter activity for each miRNA polymorphisms in the present study.

This study has several limitations. First, it is not yet clear which genetic polymorphisms predict the ischemic stroke phenotypes. This study population included only Korean individuals, and our findings need to be validated in other ethnic groups. Second, the patient population included in the subgroup analyses was relatively small. Future studies would need to include >1,000 individuals from an ethnically homogeneous population. As Koreans have a low degree of interracial marriage, this should be sufficient to provide reliable data. Third, the controls included in our study were not subjected to stringent inclusion criteria, as the enrollment rate was relatively low compared to that of stroke patients. Therefore, we could not clearly identify the causal effects of vascular risk factors in these subjects. Lastly, our results cannot be extrapolated to other ethnic groups, as racial variations in the allelic frequency could produce different results.

In conclusion, in this study, we identified a significant association between the miR-150G>A polymorphism and ischemic stroke in Korean individuals. This study demonstrates that the miR-150GA genotype occurs at a higher frequency in stroke patients, which suggests that miR-150 may play a role in the prevalence of ischemic stroke. Therefore, our findings suggest that miR-150 polymorphisms may contribute to ischemic stroke and may potentially act as biomarkers for the diagnosis and the risk of ischemic stroke. To the best of our knowledge, this is the first study to evaluate the association between miRNA polymorphisms (miR-34aC>A, miR-130aC>T, miR-150G>A and miR-155T>G) and ischemic stroke in a Korean population. Therefore, further studies on miRNA polymorphisms in other racial or ethnic populations are required in order to fully elucidate their role in the risk of ischemic stroke.

Acknowledgements

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References