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

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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* rs73056059G>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-150*GA 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-34a*C>A, *miR-130a*C>T, *miR-150*G>A and *miR-155*T>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 (miRNAs or miRs) 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-

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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 sujbects 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 cardio-embolism (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 AGC-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 *Ban*II restriction endonuclease (New England Biolabs, Beverly, MA, USA) at 37˚C for 16 h and detected using 3.5% 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 GGC CTA GAG CTC TGC 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 *Nla*III (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 GGA AGT G-3' (forward) and 5'-CCT CTG GAG TCC ACA CTC CAT-3' (reverse). DNA was amplified over 35 cycles (denaturation at 95˚C for 30 sec, annealing at 60˚C for 35 sec, and extension at 72˚C for 40 sec). The products were digested with *Bcc*I (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 TTC TAC 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 *Tsp*45I (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-

Table I. Baseline characteristics between patients with ischemic stroke and the control subjects.

Characteristic	Controls (n=404)	Stroke patients (n=596)	P-value[a]
Male (%)	173 (42.8)	254 (42.6)	0.953
Age (years, mean ± SD)	63.66±10.47	63.67±10.42	0.987
Smoking (%)	138 (34.2)	211 (35.4)	0.704
Hypertension (%)	169 (41.8)	376 (63.1)	0.0002
Diabetes mellitus (%)	54 (13.4)	161 (27.0)	<0.0001
Hyperlipidemia (%)	95 (23.5)	179 (30.0)	0.070
Homocysteine (μmol/l, mean ± SD)	10.12±4.21	11.26±6.78	0.003
Folate (nmol/l, mean ± SD)	8.88±7.99	7.06±5.29	<0.0001
Vitamin B$_{12}$ (pg/ml, mean ± SD)	746.18±670.06	747.46±624.35	0.976
Total cholesterol (mg/dl, mean ± SD)	193.70±37.59	189.88±40.79	0.137
Triglyceride (mg/dl, mean ± SD)	147.74±90.39	152.58±114.39	0.480
PLT (10^3/ml, mean ± SD)	243.06±67.51	248.19±87.73	0.324
PT (sec, mean ± SD)	11.77±0.79	11.91±3.08	0.440
aPTT (sec, mean ± SD)	33.34±18.61	30.46±4.36	0.001
Fibrinogen (mg/dl, mean ± SD)	397.83±119.62	426.93±132.40	0.018
Antithrombin (%, mean ± SD)	94.36±43.88	93.36±18.67	0.685
BUN (mg/dl, mean ± SD)	15.93±5.02	16.29±7.80	0.419
Uric acid (mg/dl, mean ± SD)	4.68±1.47	4.69±1.67	0.923

[a]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.

rium deviations and genotype and allele frequency differences between groups were assessed using χ^2 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, and the significance of differences between groups was assessed using the log-rank test. Cox regression models were used to analyze the independent prognostic importance of various markers, and results were adjusted for age, gender, diabetes mellitus, hypertension, hyperlipidemia and smoking. Hazard ratios (HRs) are presented with the 95% CI. We also performed *in silico* analysis to identify potential transcription factor binding sites in miRNA promoter regions using Alibaba 2.1 (Grabe 2002), an online bio-informatics tool.

Results

The demographic characteristics and clinical variables of the ischemic stroke and control groups are shown in Table I. When comparing the ischemic stroke group with the control group, the stroke patients had a significantly higher incidence of hypertension and diabetes, significantly higher homocysteine and fibrinogen levels, and significantly lower folate levels and aPTT (all P<0.05) (Table I).

We investigated 4 miRNA polymorphisms, *miR-34a* rs6577555C>A, *miR-130a* rs731384C>T, *miR-150* rs73056059G>A and *miR-155* rs767649T>A, and their associations with ischemic stroke. All the observed polymorphism frequencies were consistent with the Hardy-Weinberg equilibrium. 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-150*GA genotype (GG vs. AA: AOR, 1.922; 95% CI, 1.003-3.681). CE was also significantly associated with *miR-150* (GG vs. AA: AOR, 2.996; 95% CI, 1.293-6.939) (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-34a*C>A, *miR-130a*C>T, and *miR-155*T>A polymorphisms did not differ significantly between the stroke patients and the control subjects.

We performed stratified analyses according to age, gender, hypertension, diabetes mellitus, hyperlipidemia, smoking, folate levels and homocysteine levels. The *miR-34a*CA+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-150*GA+AA 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-34a*CA+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 II. Comparison of genotype frequencies of microRNA polymorphisms between ischemic stroke subtype and the controls.

Characteristics	Controls (n=404)	Stroke patients (n=596)	AOR (95% CI)[a]	P-value	P-value[b]	LAD (n=202)	AOR (95% CI)[a]	P-value	P-value[b]	SVD (n=143)	AOR (95% CI)[a]	P-value	P-value[b]	CE (n=57)	AOR (95% CI)[a]	P-value	P-value[b]
miR-34a																	
rs6577555C>A																	
CC	239 (59.2)	323 (54.2)	1.000 (reference)			107 (53.0)	1.000 (reference)			75 (52.4)	1.000 (reference)			34 (59.6)	1.000 (reference)		
CA	143 (35.4)	229 (38.4)	1.211 (0.915-1.603)	0.181	0.362	82 (40.6)	1.326 (0.911-1.930)	0.140	0.280	58 (40.6)	1.294 (0.852-1.967)	0.227	0.772	19 (33.3)	0.987 (0.537-1.813)	0.965	0.965
AA	22 (5.4)	44 (7.4)	1.384 (0.787-2.431)	0.259	0.389	13 (6.4)	1.347 (0.622-2.919)	0.450	0.675	10 (7.0)	1.244 (0.525-2.949)	0.621	0.621	4 (7.0)	1.432 (0.444-4.617)	0.548	0.822
Dominant			1.226 (0.938-1.602)	0.136	0.276		1.329 (0.927-1.905)	0.122	0.244		1.292 (0.864-1.932)	0.212	0.552		1.032 (0.581-1.834)	0.915	0.915
Recessive			1.252 (0.718-2.183)	0.429	0.562		1.192 (0.563-2.522)	0.646	0.969		1.123 (0.494-2.552)	0.782	0.782		1.277 (0.412-3.952)	0.672	0.672
miR-130a																	
rs731384C>T																	
CC	328 (81.2)	479 (80.4)	1.000 (reference)			162 (80.2)	1.000 (reference)			116 (81.1)	1.000 (reference)			45 (78.9)	1.000 (reference)		
CT	74 (18.3)	110 (18.5)	1.008 (0.715-1.420)	0.966	0.966	39 (19.3)	1.095 (0.696-1.723)	0.696	0.696	24 (16.8)	0.925 (0.545-1.570)	0.772	0.772	10 (17.5)	0.975 (0.465-2.044)	0.947	0.947
TT	2 (0.5)	7 (1.2)	1.611 (0.309-8.412)	0.572	0.572	1 (0.5)	1.079 (0.095-12.24)	0.951	0.951	3 (2.1)	4.006 (0.616-26.050)	0.146	0.219	2 (3.5)	6.229 (0.835-46.48)	0.074	0.222
Dominant			1.027 (0.733-1.439)	0.879	0.879		1.091 (0.696-1.709)	0.704	0.939		1.012 (0.608-1.686)	0.962	0.962		1.124 (0.562-2.248)	0.742	0.915
Recessive			1.630 (0.312-8.519)	0.562	0.562		0.961 (0.085-10.84)	0.974	0.974		3.907 (0.604-25.28)	0.153	0.230		5.919 (0.797-43.97)	0.082	0.246
miR-150																	
rs73056059G>A																	
GG	380 (94.1)	544 (91.3)	1.000 (reference)			181 (89.6)	1.000 (reference)			132 (92.3)	1.000 (reference)			48 (84.2)	1.000 (reference)		
GA	24 (5.9)	52 (8.7)	1.485 (0.881-2.504)	0.138	0.362	21 (10.4)	**1.922 (1.003-3.681)**	0.049	0.196	11 (7.7)	1.223 (0.558-2.683)	0.615	0.772	9 (15.8)	**2.996 (1.293-6.939)**	0.011	0.044
AA	0 (0.0)	0 (0.0)	N/A	N/A	N/A	0 (0.0)	N/A	N/A	N/A	0 (0.0)	N/A	N/A	N/A	0 (0.0)	N/A	N/A	N/A
Dominant			1.485 (0.881-2.504)	0.138	0.276		**1.922 (1.003-3.681)**	0.049	0.196		1.223 (0.558-2.683)	0.615	0.820		**2.996 (1.293-6.939)**	0.011	0.044
Recessive			N/A	N/A	N/A		N/A	N/A	N/A		N/A	N/A	N/A		N/A	N/A	N/A
miR-155																	
rs767649T>A																	
TT	117 (29.0)	167 (28.0)	1.000 (reference)			58 (28.7)	1.000 (reference)			46 (32.2)	1.000 (reference)			13 (22.8)	1.000 (reference)		
TA	191 (47.3)	311 (52.2)	1.141 (0.835-1.558)	0.409	0.545	105 (52.0)	1.168 (0.766-1.781)	0.472	0.629	69 (48.3)	0.873 (0.548-1.388)	0.565	0.772	34 (59.6)	1.508 (0.753-3.019)	0.246	0.492
AA	96 (23.8)	118 (19.8)	0.794 (0.546-1.155)	0.228	0.389	39 (19.3)	0.710 (0.420-1.200)	0.201	0.603	28 (19.6)	0.638 (0.360-1.131)	0.124	0.219	10 (17.5)	0.932 (0.385-2.258)	0.876	0.876
Dominant			1.024 (0.765-1.370)	0.876	0.879		1.006 (0.676-1.496)	0.977	0.977		0.786 (0.509-1.213)	0.276	0.552		1.280 (0.657-2.492)	0.468	0.915
Recessive			0.728 (0.529-1.002)	0.052	0.156		0.676 (0.435-1.050)	0.082	0.246		0.651 (0.395-1.074)	0.093	0.230		0.659 (0.317-1.368)	0.263	0.395

[a]Adjusted by age, gender, hypertension, diabetes mellitus, hyperlipidemia and smoking; [b]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.

Variables[b]	miR-34a rs6577555CA+AA			miR-130a rs731384CT+TT			miR-150 rs73056059GA+AA			miR-155 rs767649TA		
	AOR (95% CI)[a]	P-value	P-value[b]	AOR (95% CI)[a]	P-value	P-value[b]	AOR (95% CI)[a]	P-value	P-value[b]	AOR (95% CI)[a]	P-value	P-value[b]
Age												
<63	1.079 (0.720-1.617)	0.713	0.911	1.030 (0.608-1.746)	0.911	0.911	1.914 (0.848-4.318)	0.118	0.472	1.680 (1.037-2.722)	0.035	0.140
≥63	**1.443 (1.010-2.062)**	**0.044**	0.176	1.038 (0.673-1.601)	0.867	0.867	1.245 (0.628-2.469)	0.530	0.706	0.872 (0.576-1.319)	0.516	0.706
Gender												
Male	1.016 (0.672-1.535)	0.941	0.941	0.978 (0.577-1.657)	0.934	0.941	1.628 (0.733-3.616)	0.232	0.464	1.026 (0.621-1.695)	0.921	0.921
Female	1.459 (1.026-2.076)	0.036	0.144	1.098 (0.708-1.703)	0.677	0.677	1.360 (0.679-2.725)	0.386	0.514	1.221 (0.814-1.830)	0.334	0.514
Hypertension												
No	1.163 (0.793-1.705)	0.439	0.735	1.009 (0.618-1.649)	0.970	0.970	2.009 (0.939-4.297)	0.072	0.288	1.238 (0.798-1.922)	0.341	0.682
Yes	1.416 (0.972-2.063)	0.070	0.14	1.067 (0.672-1.695)	0.784	0.784	1.188 (0.591-2.387)	0.628	0.784	1.067 (0.680-1.674)	0.778	0.778
Diabetes mellitus												
No	**1.360 (1.013-1.827)**	**0.041**	0.164	1.028 (0.710-1.487)	0.885	0.885	1.514 (0.856-2.677)	0.154	0.229	1.044 (0.740-1.473)	0.806	0.920
Yes	0.962 (0.508-1.823)	0.906	0.906	1.103 (0.491-2.480)	0.812	0.906	1.556 (0.424-5.704)	0.505	0.906	1.761 (0.835-3.716)	0.137	0.548
Hyperlipidemia												
No	1.328 (0.974-1.810)	0.073	0.264	1.017 (0.691-1.497)	0.933	0.933	1.057 (0.586-1.907)	0.853	0.933	1.096 (0.759-1.582)	0.625	0.998
Yes	1.100 (0.645-1.877)	0.726	0.828	1.078 (0.547-2.124)	0.828	0.828	**6.060 (1.358-27.04)**	**0.018**	0.072	1.379 (0.754-2.523)	0.297	0.594
Smoking												
No	1.160 (0.834-1.612)	0.378	0.645	1.163 (0.763-1.773)	0.484	0.645	1.143 (0.612-2.137)	0.675	0.675	1.300 (0.886-1.906)	0.180	0.720
Yes	1.460 (0.923-2.308)	0.105	0.144	0.890 (0.508-1.557)	0.682	0.682	2.604 (0.936-7.241)	0.067	0.144	0.846 (0.485-1.473)	0.554	0.554
Folate[c]												
>3.56 nmol/l	1.228 (0.919-1.641)	0.165	0.330	1.071 (0.749-1.531)	0.707	0.707	1.319 (0.757-2.299)	0.328	0.437	1.190 (0.847-1.672)	0.317	0.437
≤3.56 nmol/l	0.960 (0.411-2.244)	0.925	0.925	1.795 (0.466-6.917)	0.395	0.592	N/A	N/A	N/A	0.984 (0.360-2.689)	0.974	0.974
Homocysteine[d]												
<13.92 µmol/l	1.184 (0.889-1.579)	0.249	0.332	0.994 (0.698-1.415)	0.973	0.973	1.422 (0.808-2.502)	0.223	0.332	1.180 (0.846-1.647)	0.331	0.621
≥13.92 µmol/l	1.858 (0.868-3.975)	0.110	0.44	2.068 (0.614-6.969)	0.241	0.482	1.929 (0.467-7.966)	0.364	0.485	0.959 (0.362-2.545)	0.933	0.933

[a]The adjusted odds ratio on the basis of risk factors, such as age, gender, hypertension, diabetes mellitus, hyperlipidemia and smoking; [b]false discovery rate-adjusted P-value for multiple hypotheses testing using the Benjamini-Hochberg method; [c]folate 3.56 nmol/l was the lower 15% cut-off folate level in ischemic stroke patients and controls; [d]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.

Characteristics	miR-34a rs6577555CC	miR-34a rs6577555CA+AA	miR-130a rs731384CC	miR-130a rs731384CT+TT	miR-150 rs73056059GG	miR-150 rs73056059GA+AA	miR-155 rs767649TT	miR-155 rs767649TA
Male	1.000 (reference)	1.010 (0.668-1.527)	1.000 (reference)	0.978 (0.578-1.656)	1.000 (reference)	1.614 (0.725-3.589)	1.000 (reference)	1.026 (0.621-1.695)
Female	0.800 (0.523-1.225)	1.437 (0.897-2.301)	1.067 (0.745-1.530)	1.169 (0.699-1.953)	1.034 (0.744-1.437)	1.737 (0.804-3.753)	0.895 (0.494-1.622)	1.083 (0.619-1.893)
Age <63	1.000 (reference)	1.079 (0.720-1.617)	1.000 (reference)	1.030 (0.608-1.746)	1.000 (reference)	1.914 (0.848-4.318)	1.000 (reference)	1.680 (1.037-2.722)
Age ≥63	0.790 (0.546-1.145)	1.158 (0.779-1.723)	0.864 (0.635-1.176)	0.882 (0.555-1.404)	0.876 (0.658-1.167)	1.184 (0.584-2.398)	1.404 (0.828-2.379)	1.098 (0.672-1.795)
Without hypertension	1.000 (reference)	1.154 (0.787-1.694)	1.000 (reference)	1.005 (0.616-1.639)	1.000 (reference)	2.013 (0.939-4.317)	1.000 (reference)	1.238 (0.798-1.922)
With hypertension	2.157 (1.517-3.069)	**3.088 (2.089-4.566)**	2.300 (1.711-3.093)	**2.390 (1.483-3.850)**	2.385 (1.808-3.146)	**2.871 (1.416-5.824)**	2.697 (1.612-4.511)	2.756 (1.773-4.285)
Without diabetes mellitus	1.000 (reference)	1.357 (1.011-1.823)	1.000 (reference)	1.023 (0.707-1.479)	1.000 (reference)	1.513 (0.856-2.675)	1.000 (reference)	1.044 (0.740-1.473)
With diabetes mellitus	2.452 (1.550-3.879)	2.352 (1.398-3.957)	2.096 (1.423-3.088)	**2.205 (1.043-4.661)**	2.115 (1.476-3.032)	3.161 (0.883-11.31)	1.612 (0.852-3.050)	**2.945 (1.659-5.228)**
Without hyperlipidemia	1.000 (reference)	1.323 (0.970-1.804)	1.000 (reference)	1.017 (0.691-1.497)	1.000 (reference)	1.057 (0.586-1.908)	1.000 (reference)	1.096 (0.759-1.582)
With hyperlipidemia	1.463 (0.974-2.196)	**1.570 (1.010-2.443)**	1.324 (0.945-1.857)	1.409 (0.754-2.634)	1.214 (0.887-1.662)	**7.215 (1.655-31.45)**	1.377 (0.795-2.387)	**1.734 (1.049-2.865)**
Non-smoker	1.000 (reference)	1.158 (0.833-1.610)	1.000 (reference)	1.159 (0.761-1.767)	1.000 (reference)	1.140 (0.610-2.130)	1.000 (reference)	1.300 (0.886-1.906)
Smoker	0.868 (0.566-1.330)	**1.651 (1.023-2.665)**	1.215 (0.843-1.750)	1.132 (0.650-1.971)	1.066 (0.765-1.486)	**3.594 (1.267-10.20)**	1.570 (0.848-2.907)	1.303 (0.770-2.205)
Folate >3.56 nmol/l[a]	1.000 (reference)	1.227 (0.918-1.640)	1.000 (reference)	1.071 (0.750-1.531)	1.000 (reference)	1.322 (0.758-2.304)	1.000 (reference)	1.190 (0.847-1.672)
Folate ≤3.56 nmol/l	3.780 (2.022-7.066)	3.489 (1.866-6.525)	3.138 (1.946-5.061)	**4.702 (1.341-16.49)**	3.078 (1.954-4.848)	N/A	3.731 (1.563-8.906)	3.652 (1.857-7.182)
Homocysteine <13.92 μmol/l[b]	1.000 (reference)	1.182 (0.887-1.575)	1.000 (reference)	0.996 (0.699-1.418)	1.000 (reference)	1.415 (0.803-2.493)	1.000 (reference)	1.180 (0.846-1.647)
Homocysteine ≥13.92 μmol/l	1.308 (0.763-2.243)	**2.230 (1.236-4.024)**	1.437 (0.936-2.207)	2.788 (0.891-8.718)	1.504 (0.994-2.274)	3.004 (0.804-11.23)	1.627 (0.697-3.797)	1.327 (0.742-2.372)

[a]Folate 3.56 nmol/l was the lower 15% cut-off folate level in ischemic stroke patients and controls; [b]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.

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

Characteristics	Overall (2n=2000)	Controls (2n=808)	Stroke patients (2n=1192)	OR (95% CI)	P-value[a]
*miR-34a*C>A/*miR-130a*C>T/*miR-150*G>A/*miR-155*T>A					
C-C-G-T	0.341	0.341	0.344	1.000 (reference)	
C-C-G-A	0.308	0.330	0.292	0.878 (0.704-1.095)	0.260
C-C-A-T	0.013	0.014	0.008	0.610 (0.255-1.456)	0.268
C-C-A-A	0.012	0.011	0.015	1.341 (0.594-3.030)	0.552
C-T-G-T	0.037	0.042	0.033	0.789 (0.487-1.278)	0.384
C-T-G-A	0.033	0.030	0.036	1.202 (0.713-2.026)	0.516
C-T-A-T	0.003	0.001	0.004	3.354 (0.390-28.88)	0.410
C-T-A-A	0.001	0.000	0.002	3.356 (0.160-70.22)	0.519
A-C-G-T	0.119	0.113	0.119	1.047 (0.772-1.419)	0.816
A-C-G-A	0.098	0.092	0.104	1.124 (0.811-1.557)	0.510
A-C-A-T	0.000	0.000	0.005	8.725 (0.489-155.6)	0.086
A-C-A-A	0.008	0.004	0.009	2.236 (0.610-8.200)	0.263
A-T-G-T	0.022	0.016	0.028	1.703 (0.880-3.294)	0.121
A-T-G-A	0.005	0.008	0.000	**0.052 (0.003-0.921)**	**0.004**
A-T-A-A	0.000	0.000	0.002	3.356 (0.160-70.22)	0.519
*miR-34a*C>A/*miR-130a*C>T/*miR-150*G>A					
C-C-G	0.650	0.670	0.637	1.000 (reference)	
C-C-A	0.024	0.025	0.023	0.964 (0.535-1.737)	1.000
C-T-G	0.070	0.072	0.069	1.010 (0.709-1.438)	1.000
C-T-A	0.004	0.001	0.006	4.999 (0.613-40.77)	0.150
A-C-G	0.216	0.205	0.222	1.143 (0.914-1.429)	0.258
A-C-A	0.010	0.004	0.015	**4.285 (1.255-14.62)**	**0.013**
A-T-G	0.027	0.023	0.029	1.315 (0.744-2.325)	0.399
*miR-34a*C>A/*miR-150*G>A					
C-G	0.719	0.743	0.704	1.000 (reference)	
C-A	0.029	0.026	0.030	1.226 (0.708-2.122)	0.496
A-G	0.243	0.228	0.252	1.170 (0.947-1.445)	0.149
A-A	0.009	0.004	0.014	**3.814 (1.106-13.15)**	**0.032**
*miR-150*G>A/*miR-155*T>A					
G-T	0.520	0.512	0.528	1.000 (reference)	
G-A	0.443	0.459	0.429	0.904 (0.754-1.085)	0.285
A-T	0.016	0.014	0.014	0.876 (0.410-1.870)	0.845
A-A	0.023	0.015	0.030	**1.970 (1.013-3.831)**	**0.049**

95% CI, 95% confidence interval; OR, odd ratios; [a]Fisher's exact test. MDR, multifactor dimensionality reduction. Values in bold font indicate statistical significance.

(AOR, 2.230; 95% CI, 1.236-4.024). 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, 1.267-10.20). 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 VI. Combined genotype analysis for the microRNA polymorphisms in patients with ischemic stroke and controls.

Genotype	Controls (n=404)	Stroke patients (n=596)	AOR (95% CI)[a]	P-value[b]
*miR-34a*C>A/*miR-130a*C>T				
CC-CC	193 (47.8)	260 (43.6)	1.000 (reference)	
CC-CT	45 (11.1)	57 (9.6)	0.946 (0.601-1.488)	0.809
CC-TT	1 (0.2)	6 (1.0)	4.979 (0.566-43.81)	0.148
CA-CC	119 (29.5)	187 (31.4)	1.231 (0.904-1.675)	0.187
CA-CT	23 (5.7)	41 (6.9)	1.245 (0.700-2.214)	0.456
CA-TT	1 (0.2)	1 (0.2)	0.816 (0.050-13.29)	0.887
AA-CC	16 (4.0)	32 (5.4)	1.405 (0.721-2.740)	0.318
AA-CT	6 (1.5)	12 (2.0)	2.201 (0.752-6.445)	0.150
*miR-34a*C>A/*miR-150*G>A				
CC-GG	220 (54.5)	297 (49.8)	1.000 (reference)	
CC-GA	19 (4.7)	26 (4.4)	1.036 (0.543-1.977)	0.915
CA-GG	140 (34.7)	207 (34.7)	1.134 (0.851-1.513)	0.391
CA-GA	3 (0.7)	22 (3.7)	**5.470 (1.580-18.93)**	**0.007**
AA-GG	20 (5.0)	40 (6.7)	1.570 (0.869-2.836)	0.135
AA-GA	2 (0.5)	4 (0.7)	1.329 (0.220-8.028)	0.757
*miR-34a*C>A/*miR-155*T>A				
CC-TT	64 (15.8)	90 (15.1)	1.000 (reference)	
CC-TA	115 (28.5)	166 (27.9)	1.081 (0.706-1.653)	0.721
CC-AA	60 (14.9)	67 (11.2)	0.834 (0.508-1.367)	0.471
CA-TT	49 (12.1)	63 (10.6)	1.055 (0.624-1.784)	0.841
CA-TA	65 (16.1)	120 (20.1)	1.428 (0.891-2.287)	0.139
CA-AA	29 (7.2)	46 (7.7)	1.020 (0.561-1.855)	0.949
AA-TT	4 (1.0)	14 (2.3)	3.351 (0.965-11.64)	0.057
AA-TA	11 (2.7)	25 (4.2)	1.892 (0.820-4.366)	0.135
AA-AA	7 (1.7)	5 (0.8)	0.347 (0.091-1.317)	0.120
*miR-130a*C>T/*miR-150*G>A				
CC-GG	307 (76.0)	438 (73.5)	1.000 (reference)	
CC-GA	21 (5.2)	41 (6.9)	1.308 (0.742-2.306)	0.354
CT-GG	71 (17.6)	101 (16.9)	0.965 (0.679-1.371)	0.841
CT-GA	3 (0.7)	9 (1.5)	2.292 (0.590-8.897)	0.231
TT-GG	2 (0.5)	5 (0.8)	1.660 (0.311-8.879)	0.553
*miR-130a*C>T/*miR-155*T>A				
CC-TT	91 (22.5)	135 (22.7)	1.000 (reference)	
CC-TA	156 (38.6)	243 (40.8)	1.057 (0.745-1.499)	0.757
CC-AA	81 (20.0)	101 (16.9)	0.747 (0.493-1.131)	0.168
CT-TT	26 (6.4)	29 (4.9)	0.718 (0.384-1.344)	0.301
CT-TA	33 (8.2)	64 (10.7)	1.217 (0.718-2.062)	0.465
CT-AA	15 (3.7)	17 (2.9)	0.747 (0.345-1.618)	0.459
TT-TA	2 (0.5)	4 (0.7)	1.395 (0.245-7.949)	0.708
*miR-150*G>A/*miR-155*T>A				
GG-TT	109 (27.0)	161 (27.0)	1.000 (reference)	
GG-TA	183 (45.3)	273 (45.8)	1.008 (0.730-1.392)	0.962
GG-AA	88 (21.8)	110 (18.5)	0.796 (0.540-1.173)	0.248
GA-TT	8 (2.0)	6 (1.0)	0.570 (0.181-1.799)	0.338
GA-TA	8 (2.0)	38 (6.4)	**3.265 (1.426-7.474)**	**0.005**
GA-AA	8 (2.0)	8 (1.3)	0.519 (0.176-1.534)	0.236

[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.

Characteristics	Vitamin B12 (pg/ml) Mean ± SD	P-value[a]	Fibrinogen (mg/dl) Mean ± SD	P-value[a]	Antithrombin(%) Mean ± SD	P-value[a]	aPTT (sec) Mean ± SD	P-value[a]	PT (sec) Mean ± SD	P-value[a]	PLT(10³/ml) Mean ± SD	P-value[a]
miR-34a rs6577555C>A												
CC	768.51±716.83	0.990	424.43±132.54	0.850	92.85±18.53	0.836[b]	31.54±13.80	0.714	11.93±3.32	0.595	245.87±81.18	0.929
CA	713.18±498.85		416.72±129.65		92.44±17.86		31.46±7.57		11.75±0.84		245.88±73.65	
AA	752.24±692.82		415.26±117.21		105.02±70.81		30.25±3.80		11.92±0.97		249.82±105.45	
Dominant (CC vs. CA+AA)	719.16±532.37	0.226[c]	416.49±127.54	0.430	94.46±32.87	0.770[c]	31.27±7.12	0.508[c]	11.77±0.86	0.647[c]	246.47±79.10	0.908
Recessive (CC+CA vs. AA)	752.24±692.82	0.945	415.26±117.21	0.754	105.02±70.81	0.557[c]	30.25±3.80	0.614[c]	11.92±0.97	0.304[c]	249.82±105.45	0.771[c]
miR-130a rs731384C>T												
CC	749.11±674.55	0.738	411.65±124.83	**<0.001**	93.69±27.86	0.860	31.54±12.24	0.789	11.87±2.81	0.957	244.89±71.00	0.943[b]
CT	746.52±502.57		462.34±147.50		93.30±15.50		30.98±6.55		11.84±0.91		246.99±87.54	
TT	563.89±167.02		429.80±94.44		88.00±10.56		29.87±4.18		11.64±0.61		339.78±331.57	
Dominant (CC vs. CT+TT)	738.01±493.35	0.848[c]	461.05±145.66	0.0009[c]	93.06±15.31	0.952[c]	30.91±6.44	0.837[c]	11.83±0.89	0.564[c]	251.38±111.10	0.874[c]
Recessive (CC+CT vs. TT)	563.89±167.02	0.207[c]	429.80±94.44	0.878	88.00±10.56	0.389[c]	29.87±4.18	0.602[c]	11.64±0.61	0.648[c]	339.78±331.57	0.790[c]
miR-150 rs73056059G>A												
GG	748.05±660.94	0.738	418.79±129.21	0.116	93.59±26.44	0.948	31.49±11.75	0.563	11.86±2.65	0.698	244.44±66.30	**0.002**
GA	733.68±365.56		444.18±141.02		93.36±19.96		30.65±5.18		11.98±1.06		266.53±58.58	
AA	N/A		N/A		N/A		N/A		N/A		N/A	
Dominant (GG vs. GA+AA)	733.68±365.56	0.298[c]	444.18±141.02	0.163	93.36±19.96	0.808[c]	30.65±5.18	0.751[c]	11.98±1.06	0.219[c]	266.53±58.58	**0.002**
Recessive (GG+GA vs. AA)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
miR-155 rs767649T>A												
TT	725.86±550.21	0.287	415.63±129.13	0.055	97.42±39.69	**0.049**	31.20±6.75	0.757	11.74±0.75	0.356[b]	242.93±65.56	0.632
TA	772.02±713.53		432.23±134.92		91.92±17.99		31.70±14.51		11.78±1.02		248.47±91.67	
AA	716.23±580.51		400.34±117.63		92.29±16.25		31.04±6.74		12.23±5.27		244.90±68.49	
Dominant (TT vs. TA+AA)	755.38±676.66	0.620[c]	423.01±130.84	0.505	92.03±17.47	0.157[c]	31.50±12.73	0.936[c]	11.91±2.98	0.700[c]	247.40±85.37	0.319[c]
Recessive (TT+TA vs. AA)	716.23±580.51	**0.030[c]**	400.34±117.63	**0.036**	92.29±16.25	0.644[c]	31.04±6.74	0.445[c]	12.23±5.27	0.151[c]	244.90±68.49	0.570[c]

[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.
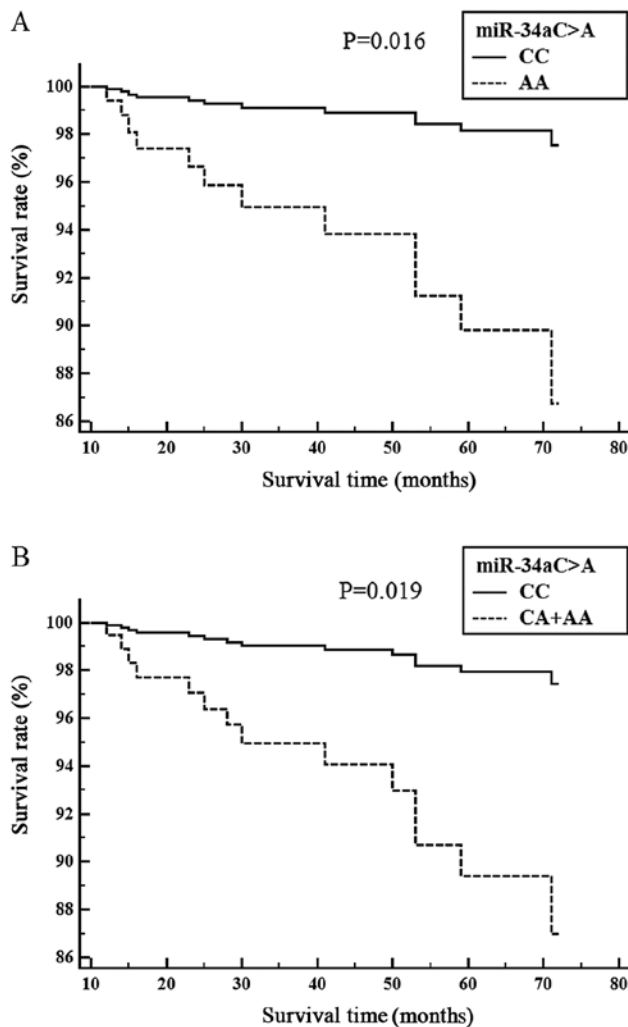
A

B

Figure 1. Cox proportional hazards regression of survival of patients with small-vessel disease (SVD) according to *miR-34a*C>A polymorphisms. (A) Survival curves for the *miR-34a*C>A polymorphism (CC vs. CA) (P=0.016). (B) Survival curves for the *miR-34a*C>A polymorphism (CC vs. CA+AA) (P=0.019).

*miR-34a*CA/*miR-150*GA genotype (AOR, 5.470; 95% CI, 1.580-18.932) and the *miR-150*GA/*miR-155*TA genotype (AOR, 3.265; 95% CI, 1.426-7.474) were both associated with an 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±17.99; 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
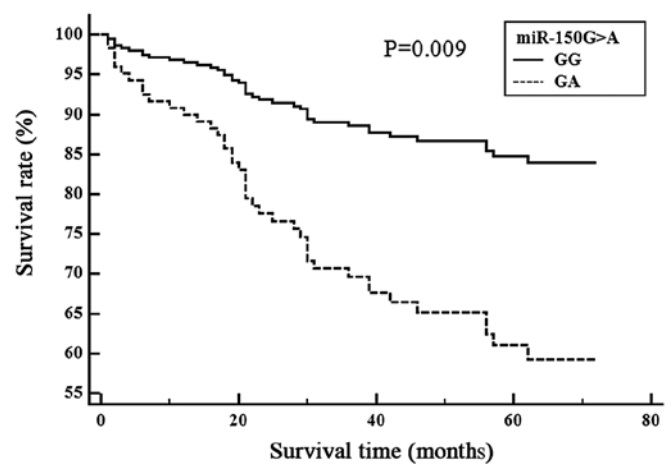

Figure 2. Cox proportional hazards regression of survival of patients with large-artery disease (LAD) according to *miR-150*G>A polymorphisms. Survival curves for the *miR-150*G>A polymorphism (GG vs. GA) (P=0.009).

subgroup (CC vs. CA, P=0.016) (Fig. 1A), (CC vs. CA+AA, P=0.019) (Fig. 1B). The *miR-150*GA 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-150*G>A polymorphism may be associated with a susceptibility for LAD and CE ischemic stroke. In the gene-environment interaction analysis, the *miR-150*GA+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-150*A allele exhibited a marked increase in ischemic stroke incidence, whereas subjects with the *miR-150*G allele had a decreased prevalence of ischemic stroke. These data were confirmed in combination analysis, where the *miR-150*GA genotype also exhibited an increased prevalence of ischemic stroke. The *miR-150*GA 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-150*GA 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), tumorigenesis (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 megakaryocytopoiesis 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-150*GA 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 casual 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-150*G>A polymorphism and ischemic stroke in Korean individuals. This study demonstrates that the *miR-150*GA 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-34a*C>A, *miR-130a*C>T, *miR-150*G>A and *miR-155*T>A) 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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