JoS JOURNAL OF STROKE

Journal of Stroke 2018;20(1):110-121
https://doi.org/10.5853/jos.2017.02586

Original Article

Association of MicroRNA Biogenesis Genes Polymorphisms with Ischemic Stroke Susceptibility and Post-Stroke Mortality

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Background and Purpose MicroRNA (miRNA) expression has been examined in multiple conditions, including various cancers, neurological diseases, and cerebrovascular diseases, particularly stroke. Existing evidence indicates that miRNA biosynthesis and function play crucial roles in ischemic stroke physiology and pathology. In this study, we selected six known polymorphisms in miRNA-biogenesis genes; *DICER* rs13078A>T, rs3742330A>G; *DROSHA* rs10719T>C, rs6877842G>C; Ran GTPase (*RAN*) rs14035C>T; exportin 5 (*XPO5*) rs11077A>C.

Methods We analyzed the associations between these polymorphisms and disease status and clinical factors in 585 ischemic stroke patients and 403 controls. Genotyping was performed with the polymerase chain reaction-restriction fragment length polymorphism method.

Results The *DICER* rs3742330A>G (AA vs. AG+GG: adjusted odds ratio [AOR], 1.360; 95% confidence interval [CI], 1.024 to 1.807; *P*=0.034) and *DROSHA* rs10719T>C polymorphisms (TT vs. CC: AOR, 2.038; 95% CI, 1.113 to 3.730; *P*=0.021) were associated with ischemic stroke prevalence. During a mean follow-up of 4.80±2.11 years, 99 (5.91%) of the stroke patients died. In multivariate Cox proportional hazard regression models, a significant association was found between *RAN* rs14035 and survival of large artery disease patients with ischemic stroke (CC vs. TT: adjusted hazard ratio, 5.978; *P*=0.015).

Conclusions An association was identified between the *DICER* and *DROSHA* polymorphisms and ischemic stroke. Specifically, polymorphisms (rs3742330 and rs10719) were more common in stroke patients, suggesting that they may be associated with an increased risk of ischemic stroke.

Keywords Polymorphism, genetic; Stroke; MicroRNA biogenesis genes; Mortality

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Received: November 6, 2017
Revised: December 30, 2017
Accepted: January 19, 2018

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Introduction

Stroke is regarded as a complex, multifactorial, polygenic disease arising from a wide number of gene-gene and gene-environment interactions.[1,2] Multiple factors including hypertension, diabetes mellitus, smoking, hyperlipidemia, and hyperhomocysteinemia are associated with a higher risk of stroke.[3,4] Hyperhomocysteinemia, in particular, has been demonstrated to be an independent risk factor for ischemic stroke in several studies involving different ethnic groups.[5,6]

MicroRNAs (miRNAs) are a class of endogenous, small, noncoding RNAs that pair with sites in 3′-untranslated regions (3′-UTRs) in mRNAs to downregulate their expression.[7-9] Previous studies have suggested that gene expression may be regulated by a small number of miRNAs.[10-13] To date, miRNA expression has been examined in patients with tumors,[12,14,15] Alzheimer's disease,[16] Parkinson's disease,[17] schizophrenia,[18] and stroke.[19-21] The evidence gathered to date indicates that miRNA biosynthesis plays crucial, physiological, and pathological roles.[22-24] Biosynthesis of miRNAs involves several miRNA biogenesis genes and occurs in multiple steps.[7] RNA polymerase II produces large primary miRNA transcripts (about 500 to 3,000 nucleotides) in the nucleus. The transcripts are processed by a multiprotein complex that includes *DROSHA* to form precursor miRNA (pre-miRNA) hairpins (about 60 to 100 nucleotides). After pre-miRNA has been exported to the cytoplasm by Ran GTPase (*RAN*) and exportin 5 (*XPO5*), it is further processed by *DICER*1, a polymerase II enzyme. Subsequently, the double-stranded miRNA duplex unwinds, forming an 18- to 24-nucleotide single-stranded, mature miRNA.[7,25-27]

The present study tested the hypothesis that there is an association between miRNA biogenesis gene polymorphism and ischemic stroke risk. The objective was to investigate associations between six known miRNA biogenesis gene polymorphisms (*DICER* 3′-UTR rs13078A>T, *DICER* 3′-UTR rs3742330A>G, *DROSHA* 3′-UTR rs10719T>C, *DROSHA* 3′-UTR rs6877842G>C, *RAN* 3′-UTR rs14035C>T, and *XPO5* 3′-UTR rs11077A>C) and ischemic stroke and its risk factors.

Methods

Ethics statement

All study protocols were reviewed and approved by the Institutional Review Board of CHA Bundang Medical Center and followed the recommendations of the Declaration of Helsinki. Study subjects were recruited from the South Korean provinces of Seoul and Gyeonggi-do between 2000 and 2008. The Institutional Review Board of CHA Bundang Medical Center approved this genetic study in June 2000 (IRB No. 2013-09-073) and informed consent was obtained from study participants.

Study population

The Department of Neurology at CHA Bundang Medical Center, CHA University, referred 585 consecutive patients with ischemic stroke. Ischemic stroke was defined as a stroke (a clinical syndrome characterized by rapidly developing clinical symptoms and signs of focal or global loss of brain function) with evidence of cerebral infarction in clinically relevant areas of the brain according to magnetic resonance imaging (MRI) scan finding. Based on clinical manifestations and neuroimaging data, two neurologists classified all ischemic strokes into four causative subtypes using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria, as follows: (1) large artery disease (LAD), characterized by an infarction lesion ≥15 mm in diameter documented by MRI, and significant (>50%) stenosis of a major brain artery or a branch cortical artery documented by cerebral angiography with symptoms associated with that arterial territory; (2) small vessel disease (SVD), characterized by an infarction lesion <15 and ≥5 mm in diameter documented using MRI, and classic lacunar syndrome without evidence of cerebral cortical dysfunction or potentially detectable cardiac sources for embolism; (3) cardioembolism (CE) or arterial occlusions presumably due to an embolus arising in the heart, as detected by cardiac evaluation; and (4) undetermined pathogenesis, in which the cause of stroke could not be determined with any degree of confidence or involved >2 causes. Single and multiple (≥2 lesions) SVD cases were distinguished via brain MRI scans. The sizes and sites of cerebral infarctions were documented using MRI only. We selected 403 control subjects that were matched for sex ratio and age (within 5 years) in accordance with the patient group (Table 1). Controls were drawn from subjects visiting our hospitals during the same period for health examinations, including biochemical testing, electrocardiograms, and brain MRIs. Control subjects did not have a recent history of cerebrovascular disease or myocardial infarction. Exclusion criteria were the same as those used for the case group, as mentioned previously.

Genotyping

DNA was extracted from leukocytes using a G-DEX II Genomic DNA Extraction kit (Intron Biotechnology, Seongnam, Korea) according to the manufacturer's instructions. The six best-studied single nucleotide polymorphisms (SNPs) in the miRNA biogenesis genes were determined through a documentary search that included 3′-UTR SNPs (*DICER* rs13078A>T, rs3742330A>G, *DROSHA* rs10719T>C, rs6877842G>C, *RAN* rs14035C>T, and *XPO5* rs11077A>C). The miRNA biogenesis gene polymorphisms

Table 1. Baseline characteristics of ischemic stroke cases and controls

Characteristic	Cases (n=585)	Controls (n=403)	*P**
Male sex	243 (41.5)	168 (41.7)	1.000
Age (yr)	62.72±10.91	62.79±10.61	0.249
Smokers	206 (35.2)	133 (33.0)	0.654
Hypertension	367 (62.7)	164 (40.7)	0.0002
Diabetes mellitus	156 (26.7)	52 (12.9)	<0.0001
Hyperlipidemia	178 (30.4)	94 (23.3)	0.069
tHcy (μmol/L)	11.18±6.81	10.06±4.20	0.004
Folate (nmol/L)	6.99±5.14	8.55±5.96	<0.0001
Vitamin B_{12} (pg/mL)	747.10±616.70	744.70±669.67	0.905
Total cholesterol (mg/dL)	191.09±40.39	193.28±37.74	0.353
Triglycerides (mg/dL)	154.39±114.25	147.24±90.61	0.592
PLT (10^3/μL)	249.22±87.57	243.10±67.35	0.240
PT (sec)	11.78±0.98	11.77±0.80	0.875
aPTT (sec)	30.47±4.43	33.42±18.58	<0.001
Fibrinogen (mg/dL)	424.74±130.82	94.45±44.03	<0.001
Antithrombin (%)	94.09±18.82	400.17±120.45	<0.001
BUN (mg/dL)	15.86±6.10	15.78±4.93	0.828
Uric acid (mg/dL)	4.67±1.55	4.64±1.44	0.733

Values are presented as number (%) or mean±SD.
tHcy. total homocysteine; PLT, platelet count; PT, prothrombin time; aPTT, activated partial thromboplastin time; BUN, blood urea nitrogen.
**P*-values were calculated using the two-sided t-test for continuous variables and the chi-square test for categorical variables.

were analyzed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The PCR conditions for miRNA biogenesis genes polymorphism analyses are presented in Supplementary Table 1. To validate RFLP findings, 30% of the PCR assays for each polymorphism were randomly selected and repeated, followed by DNA sequencing. Sequencing was performed using an ABI 3730×l DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The concordance of these quality control samples with the RFLP results was 100%.

Post-stroke mortality

To evaluate the association between miRNA biogenesis gene polymorphisms and long-term prognosis after ischemic stroke, survival time from stroke onset to death was tracked. The dates of death for each stroke patient (n=585) were ascertained using death certificates from the Korean National Statistical Office. Patients who were alive on December 31, 2013 were excluded from the study.

Statistical analysis

Genotype and allele combination frequencies in ischemic stroke cases and controls were compared using multivariate logistic regression models and Fisher exact test, respectively. Allele frequencies were calculated to identify deviations from Hardy-Weinberg equilibrium using P=0.05 as a threshold. Odds ratios, adjusted odds ratios (AORs), and 95% confidence intervals (CIs) were used to measure the strength of association between various genotypes and ischemic stroke. The association between miRNA biogenesis gene SNPs and post-stroke mortality was evaluated using Cox proportional hazard regression. The proportional hazards assumption was tested using a log(–log[survival]) plot and interaction for follow-up time in a time-dependent Cox regression model, which was found to be satisfactory. For multivariate analyses, logistic regression analyses were used to adjust for possible confounders, including age, sex, hypertension, diabetes mellitus, hyperlipidemia, and smoking. Statistical significance was accepted at the P<0.05 level.[28,29]

Results

Baseline characteristics

The demographic characteristics of the 585 stroke cases and 403 controls are presented in Table 1. Of the stroke and control samples, 41.5% and 41.7%, respectively, were men, and the mean ages of stroke cases and controls were 62.7±10.9 and 62.8±10.6 years, respectively.

https://doi.org/10.5853/jos.2017.02586

Table 2. Comparison of *DICER*, *DROSHA*, *RAN*, and *XPO5* polymorphisms between ischemic stroke patients and controls subjects

Genotype	Controls (n=403)	Cases (n=585)	COR (95% CI)	*P**	*P*†	AOR (95% CI)‡	*P*§	*P*†
DICER rs13078 A>T								
AA	360 (89.3)	527 (90.1)	1.000 (reference)			1.000 (reference)		
AT	43 (10.7)	55 (9.4)	0.874 (0.574–1.331)	0.530	0.530	0.926 (0.596–1.439)	0.733	0.733
TT	0	3 (0.5)	NA			NA	0.994	0.994
Dominant (AA vs. AT+TT)			0.921 (0.608–1.398)	0.700	0.700	0.978 (0.633–1.511)	0.920	0.920
Recessive (AA+AT vs. TT)			NA			NA	0.994	0.994
HWE-*P*	0.258	0.238						
DICER rs3742330 A>G								
AA	148 (36.7)	169 (28.9)	1.000 (reference)			1.000 (reference)		
AG	180 (44.7)	280 (47.9)	1.362 (1.020–1.820)	0.036	0.129	1.313 (0.969–1.779)	0.079	0.237
GG	75 (18.6)	136 (23.2)	1.588 (1.110–2.272)	0.011	0.043	1.459 (1.000–2.126)	0.050	0.100
Dominant (AA vs. AG+GG)			1.429 (1.090–1.872)	0.010	0.057	1.360 (1.024–1.807)	0.034	0.102
Recessive (AA+AG vs. GG)			1.325 (0.966–1.817)	0.081	0.135	1.254 (0.902–1.745)	0.178	0.356
HWE-*P*	0.125	0.337						
DROSHA rs6877842 C>G								
CC	371 (92.1)	548 (93.7)	1.000 (reference)			1.000 (reference)		
CG	31 (7.7)	36 (6.2)	0.786 (0.478–1.294)	0.344	0.503	0.785 (0.467–1.320)	0.361	0.542
GG	1 (0.2)	1 (0.2)	0.677 (0.042–10.858)	0.783	0.783	0.769 (0.046–12.813)	0.855	0.994
Dominant (CC vs. CG+GG)			0.783 (0.479–1.279)	0.328	0.394	0.784 (0.470–1.309)	0.352	0.422
Recessive (CC+CG vs. GG)			0.688 (0.043–11.038)	0.792	0.792	0.766 (0.046–12.743)	0.852	0.994
HWE-*P*	0.680	0.614						
DROSHA rs10719 T>C								
TT	228 (56.6)	304 (52.0)	1.000 (reference)			1.000 (reference)		
TC	158 (39.2)	235 (40.2)	1.116 (0.856–1.454)	0.419	0.503	1.102 (0.835–1.455)	0.492	0.590
CC	17 (4.2)	46 (7.9)	2.029 (1.134–3.633)	0.017	0.043	2.038 (1.113–3.730)	0.021	0.994
Dominant (TT vs. TC+CC)			1.204 (0.933–1.554)	0.153	0.306	1.193 (0.913–1.558)	0.196	0.294
Recessive (TT+TC vs. CC)			1.938 (1.094–3.432)	0.023	0.115	2.001 (1.106–3.621)	0.022	0.132
HWE-*P*	0.107	0.950						
RAN rs14035 C>T								
CC	240 (59.6)	369 (63.1)	1.000 (reference)			1.000 (reference)		
CT	149 (37.0)	192 (32.8)	0.838 (0.641–1.097)	0.198	0.396	0.803 (0.606–1.064)	0.127	0.254
TT	14 (3.5)	24 (4.1)	1.115 (0.566–2.198)	0.753	0.783	1.106 (0.545–2.244)	0.780	0.994
Dominant (CC vs. CT+TT)			0.862 (0.664–1.118)	0.263	0.394	0.830 (0.632–1.091)	0.181	0.294
Recessive (CC+CT vs. TT)			1.189 (0.607–2.327)	0.614	0.768	1.198 (0.597–2.403)	0.611	0.917
HWE-*P*	0.114	0.876						
XPO5 rs11077 A>C								
AA	319 (79.2)	497 (85.0)	1.000 (reference)			1.000 (reference)		
AC	79 (19.6)	87 (14.9)	0.707 (0.505–0.989)	0.043	0.129	0.707 (0.497–1.005)	0.053	0.237
CC	5 (1.2)	1 (0.2)	0.128 (0.015–1.104)	0.062	0.103	0.101 (0.011–0.951)	0.045	0.100
Dominant (AA vs. AC+CC)			0.672 (0.483–0.936)	0.019	0.057	0.669 (0.473–0.945)	0.023	0.102
Recessive (AA+AC vs. CC)			0.136 (0.016–1.171)	0.069	0.135	0.116 (0.013–1.078)	0.058	0.174
HWE-*P*	0.965	0.161						

Values are presented as number (%).
RAN, Ran GTPase; XPO5, exportin 5; COR, crude odds ratio; CI, confidence interval; AOR, adjusted odds ratio; NA, not available; HWE, Hardy-Weinberg equilibrium.
*Calculated by chi-square test according to genotype frequencies; †*P*-value calculated by false discovery rate test; ‡Odds ratios adjusted for age, sex, hypertension, diabetes mellitus, hyperlipidemia, and smoking status; §*P*-value calculated by logistics regression analysis.

Table 3. Comparison of *DICER*, *DROSHA*, *RAN*, and *XPO5* polymorphisms between ischemic stroke subtypes and controls subjects

Genotype	Controls (n=403)	Stroke: LAD (n=200)	AOR (95% CI)*	P†	P‡	Stroke: SVD (n=149)	AOR (95% CI)*	P†	P‡	Stroke: CE (n=54)	AOR (95% CI)*	P†	P‡
DICER rs13078 A>T													
AA	360 (89.3)	186 (93.0)	1.000 (reference)			130 (87.2)	1.000 (reference)			49 (90.7)	1.000 (reference)		
AT	43 (10.7)	14 (7.0)	0.597 (0.309–1.156)	0.126	0.378	19 (12.8)	1.368 (0.747–2.506)	0.310	0.465	4 (7.4)	0.621 (0.212–1.823)	0.386	0.701
TT	0	0	NA			0	NA			1 (1.9)	NA	0.995	0.996
Dominant (AA vs. AT+TT)			0.597 (0.309–1.156)	0.126	0.378		1.368 (0.747–2.506)	0.310	0.465		0.778 (0.291–2.078)	0.617	0.779
Recessive (AA+AT vs. TT)			NA				NA				NA	0.995	0.998
DICER rs3742330 A>G													
AA	148 (36.7)	62 (31.0)	1.000 (reference)			40 (26.8)	1.000 (reference)			18 (33.3)	1.000 (reference)		
AG	180 (44.7)	89 (44.5)	1.084 (0.715–1.643)	0.703	0.844	78 (52.3)	1.705 (1.073–2.710)	0.024	0.144	20 (37.0)	0.885 (0.448–1.750)	0.726	0.726
GG	75 (18.6)	49 (24.5)	1.448 (0.882–2.375)	0.143	0.600	31 (20.8)	1.412 (0.785–2.539)	0.249	0.623	16 (29.6)	1.535 (0.723–3.257)	0.264	0.792
Dominant (AA vs. AG+GG)			1.187 (0.809–1.741)	0.380	0.535		1.616 (1.041–2.509)	0.032	0.192		1.099 (0.597–2.021)	0.763	0.779
Recessive (AA+AG vs. GG)			1.385 (0.899–2.133)	0.140	0.655		1.041 (0.635–1.708)	0.873	0.993		1.681 (0.879–3.215)	0.116	0.348
DROSHA rs6877842 C>G													
CC	371 (92.1)	188 (94.0)	1.000 (reference)			143 (96.0)	1.000 (reference)			48 (88.9)	1.000 (reference)		
CG	31 (7.7)	12 (6.0)	0.777 (0.379–1.589)	0.489	0.786	5 (3.4)	0.428 (0.159–1.153)	0.093	0.279	6 (11.1)	1.416 (0.555–3.610)	0.467	0.701
GG	1 (0.2)	0	NA	0.995	0.995	1 (0.7)	3.405 (0.201–57.78)	0.396	0.660	0	NA	0.996	0.996
Dominant (CC vs. CG+GG)			0.758 (0.371–1.546)	0.446	0.535		0.505 (0.201–1.267)	0.145	0.394		1.389 (0.546–3.535)	0.491	0.779
Recessive (CC+CG vs. GG)			NA	0.995	0.995		3.438 (0.203–58.176)	0.392	0.960		NA	0.996	0.998
DROSHA rs10719 T>C													
TT	228 (56.6)	106 (53.0)	1.000 (reference)			78 (52.3)	1.000 (reference)			29 (53.7)	1.000 (reference)		
TC	158 (39.2)	82 (41.0)	1.129 (0.777–1.640)	0.524	0.786	60 (40.3)	1.089 (0.720–1.647)	0.686	0.686	18 (33.3)	0.865 (0.462–1.620)	0.650	0.726
CC	17 (4.2)	12 (6.0)	1.646 (0.717–3.778)	0.240	0.600	11 (7.4)	1.786 (0.759–4.202)	0.184	0.623	7 (13.0)	3.451 (1.264–9.422)	0.016	0.096
Dominant (TT vs. TC+CC)			1.170 (0.816–1.678)	0.392	0.535		1.156 (0.777–1.718)	0.475	0.535		1.086 (0.610–1.935)	0.779	0.779
Recessive (TT+TC vs. CC)			1.588 (0.708–3.562)	0.262	0.655		1.674 (0.730–3.838)	0.224	0.960		3.499 (1.348–9.082)	0.010	0.060
RAN rs14035 C>T													
CC	240 (59.9)	119 (59.5)	1.000 (reference)			89 (59.7)	1.000 (reference)			39 (72.2)	1.000 (reference)		
CT	149 (37.0)	74 (37.0)	0.990 (0.680–1.442)	0.958	0.958	54 (36.2)	0.852 (0.560–1.296)	0.454	0.545	13 (24.1)	0.556 (0.283–1.092)	0.088	0.360
TT	14 (3.5)	7 (3.5)	0.886 (0.327–2.397)	0.811	0.995	6 (4.0)	1.189 (0.417–3.391)	0.747	0.934	2 (3.7)	0.848 (0.180–3.985)	0.834	0.996
Dominant (CC vs. CT+TT)			0.986 (0.684–1.421)	0.938	0.938		0.879 (0.585–1.320)	0.535	0.535		0.572 (0.301–1.088)	0.088	0.264
Recessive (CC+CT vs. TT)			0.881 (0.334–2.321)	0.798	0.995		1.341 (0.480–3.742)	0.576	0.960		0.999 (0.217–4.585)	0.998	0.998

https://doi.org/10.5853/jos.2017.02586

Table 3. Continued

Genotype	Controls (n=403)	Stroke: LAD (n=200)	AOR (95% CI)*	P†	P†	Stroke: SVD (n=149)	AOR (95% CI)*	P†	P†	Stroke: CE (n=54)	AOR (95% CI)*	P†	P†
XPO5 rs11077 A>C													
AA	319 (79.2)	171 (85.5)	1.000 (reference)			125 (83.9)	1.000 (reference)			48 (88.9)	1.000 (reference)		
AC	79 (19.6)	29 (14.5)	0.665 (0.407–1.087)	0.103	0.378	24 (16.1)	0.756 (0.446–1.280)	0.297	0.465	6 (11.1)	0.490 (0.199–1.204)	0.120	0.360
CC	5 (1.2)	0	NA	0.995	0.995	0	NA	0.993	0.934	0	NA	0.995	0.996
Dominant (AA vs. AC+CC)			0.619 (0.380–1.008)	0.054	0.324		0.708 (0.420–1.196)	0.197	0.394		0.454 (0.185–1.112)	0.084	0.264
Recessive (AA+AC vs. CC)			NA	0.995	0.995		NA	0.993	0.993		NA	0.996	0.998

Values are presented as number (%).
RAN, Ran GTPase; XPO5, exportin 5; LAD, large artery disease; AOR, adjusted odds ratio; CI, confidence interval; SVD, small vessel disease; CE, cardioembolism; NA, not available.
*Odds ratios adjusted for age, sex, hypertension, diabetes mellitus, hyperlipidemia, and smoking status; †*P*-value calculated by logistics regression analysis; ‡*P*-value calculated by false discovery rate test.

Genotype frequencies of the miRNA biogenesis genes polymorphisms

Table 2 provides the genotype distributions of the six miRNA biogenesis gene polymorphisms in ischemic stroke cases and controls. The *DICER* rs3742330A>G polymorphism was associated with greater odds of ischemic stroke (AA vs. GG: AOR, 1.459; 95% CI, 1.000 to 2.126; *P*=0.050; and AA vs. AG+GG: AOR, 1.360; 95% CI, 1.024 to 1.807; *P*=0.034). The *DROSHA* rs10719T>C polymorphism was also associated with greater odds of ischemic stroke (TT vs. CC: AOR, 2.038; 95% CI, 1.113 to 3.730; *P*=0.021; and TT+TC vs. CC: AOR, 2.001; 95% CI, 1.106 to 3.621; *P*=0.022). By contrast, the *XPO5* rs11077A>C polymorphism was associated with lower odds of stroke (AA vs. CC: AOR, 0.101; 95% CI, 0.011 to 0.951; *P*=0.045; and AA vs. AC+CC: AOR, 0.669; 95% CI, 0.473 to 0.945; *P*=0.023). The frequency of the *DICER1* rs13078A>T, *DROSHA* ABI 3730×l DNA Analyzer C>G, and *RAN* rs14035C>T polymorphisms was not significantly different between stroke cases and controls. To examine whether the effect of each polymorphism was confined to a specific subtype, stroke patients were separated into three subgroups (LAD, SVD, and CE) according to TOAST classifications (Table 3). Comparisons were also performed with control subjects and single versus multiple SVD patients (Supplementary Table 2). LAD was not significantly associated with a ny of the polymorphisms examined. However, the *DICER1* rs3742330A>G polymorphism was significantly associated with SVD (AA vs. AG: AOR, 1.705; 95% CI, 1.073 to 2.710; *P*=0.024; and AA vs. AG+GG: AOR, 1.616; 95% CI, 1.041 to 2.509; *P*=0.032). In addition, the *DROSHA* rs10719T>C polymorphism was significantly associated with CE (TT vs. CC: AOR, 3.451; 95% CI, 1.264 to 9.422; *P*=0.016; and TT+TC vs. CC: AOR, 3.499; 95% CI, 1.348 to 9.082; *P*=0.010).

Combined effects of miRNA biogenesis gene polymorphisms and clinical factors

Stratified analysis of each clinical factor was performed to confirm the influence of clinical factors on the occurrence of ischemic stroke. However, no significant clinical factors were found to affect ischemic stroke risk (Supplementary Table 3). Therefore, a combined effect analysis was conducted to ascertain the effect of stroke and genotype on the prevalence of ischemic stroke. A synergistic effect was found for ischemic stroke prevalence between clinical factors (hypertension and diabetes mellitus) and miRNA biogenesis gene polymorphisms (Figure 1). The *DROSHA* rs10719 CC genotype was associated with stroke in individuals with hypertension (AOR, 4.781; 95% CI, 1.981 to 11.54). Diabetes mellitus combined with the *DROSHA* rs10719 CC genotype yielded the most significant associa-

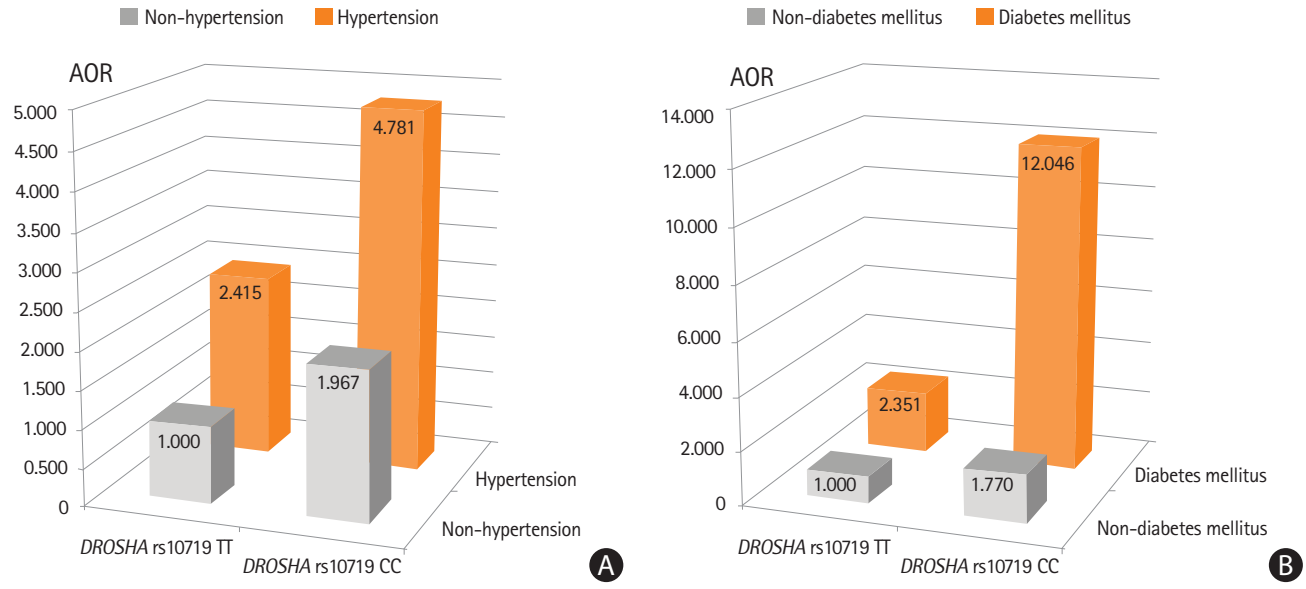

Figure 1. The effects of *DROSHA* rs10719 T>C variant on ischemic stroke development modulated by clinical factors. (A) The synergistic effect for ischemic stroke susceptibility in *DROSHA* rs10719CC with hypertension (adjusted odds ratio [AOR], 4.781), *DROSHA* rs10719CC with non-hypertension (AOR, 1.967), or *DROSHA* rs10719TT with hypertension (AOR, 2.415). (B) *DROSHA* rs10719 T>C was associated with elevated ischemic stroke prevalence, in the case of *DROSHA* rs10719CC, with diabetes mellitus (AOR, 12.046), *DROSHA* rs10719CC, with non-diabetes mellitus (AOR, 1.770), and *DROSHA* rs10719TT, with diabetes mellitus (AOR, 2.351).

tion with stroke (AOR, 12.05; 95% CI, 1.541 to 94.19). Other gene-clinical factor combinations were not significantly associated with ischemic stroke (Supplementary Tables 4-6). The effects of miRNA biogenesis gene genotypes on blood coagulation status were evaluated by measuring platelet proportion, prothrombin time, activated partial thromboplastin time (aPTT), fibrinogen, and antithrombin (Supplementary Table 7). It was found that the *DROSHA* rs10719 CC genotype was significantly associated with elevated aPTT (TT vs. CC: P=0.007; TC vs. CC: P=0.019) (Supplementary Figure 1A) and antithrombin (TC vs. CC: P=0.039) (Supplementary Figure 1B). The other coagulant factors did not exhibit any statistically significant associations with any of the tested genotypes.

Polymorphisms in miRNA biogenesis genes versus post-stroke mortality

To evaluate the association between miRNA biogenesis gene polymorphisms and post-stroke mortality, Cox regression analysis was performed on the 585 patients with total ischemic stroke according to TOAST subtype (Figure 2 and Supplementary Table 8). During a mean follow-up of 4.80±2.11 years, 99 of the stroke patients died. In the multivariate Cox proportional hazard regression models, a significant association was found between *RAN* rs14035 and survival of LAD patients with ischemic stroke (CC vs. TT: adjusted hazard ratio [HR], 5.978; P=0.015; and CC+CT vs. TT: adjusted HR, 3.946; P=0.034) (Figure 2). A significant association was also found between *RAN* rs14035 and SVD in our analysis of ischemic stroke subtypes (CC vs. TT: adjusted HR, 9.403; P=0.015; and CC+CT vs. TT: adjusted HR, 5.223; P=0.039) (Figure 2). However, survival analysis was performed by Cox proportional-hazards regression based on the stepwise method for confirming covariant effect. A stepwise Cox regression analysis of ischemic stroke-related survival is shown in Supplementary Table 9. Mortality in SVD subgroup of ischemic stroke cases was associated with age and *RAN* rs14039 polymorphism status.

Supplemental data

Gene-gene interaction analyses were performed for miRNA biogenesis gene polymorphisms to identify combinations that have synergistic effects on stroke risk (Supplementary Tables 10 and 11). Some variants and allele combinations exhibited significant associations. However, the meaning of these associations should be interpreted with caution because the sample size is rather small.

Discussion

A recent study indicated that miR-221 and miR-222 modulate the angiogenic properties of human umbilical vein endothelial cells.[30] However, the function and biosynthesis of miRNAs in endothelial cell biology remains unclear. Therefore, in this

https://doi.org/10.5853/jos.2017.02586

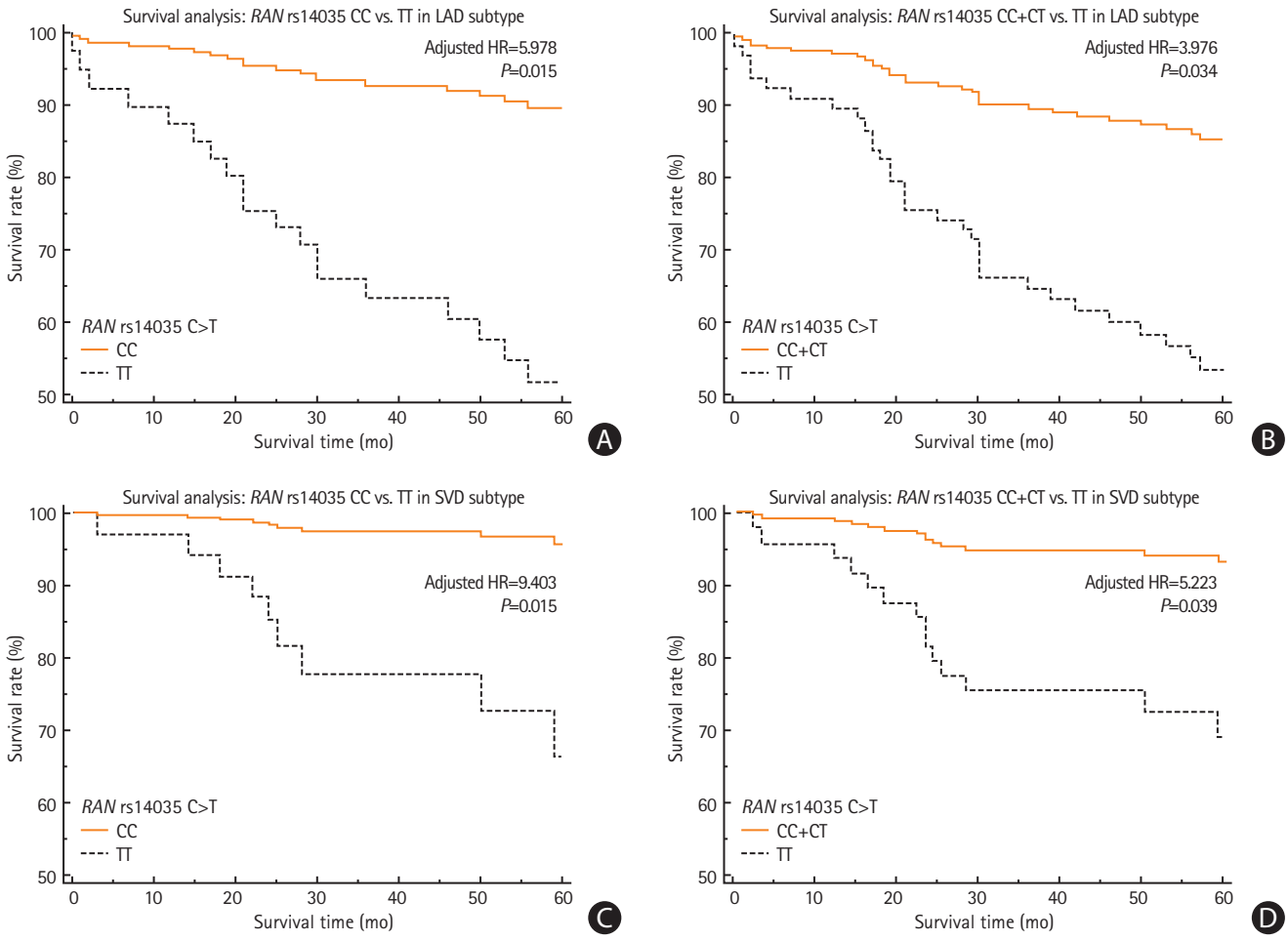

Figure 2. Survival plot from a Cox proportional hazards model with *RAN* rs14035C>T polymorphisms in ischemic stroke. Survival curve of patients grouped by large artery disease (LAD) subtype based on (A) *RAN* rs14035CC vs. *RAN* rs14035TT genotypes and (B) *RAN* rs14035CC+CT vs. *RAN* rs14035TT genotypes. In addition, survival curve of patients grouped by SVD subtype based on (C) *RAN* rs14035CC vs. *RAN* rs14035TT genotypes and (D) *RAN* rs14035CC+CT vs. *RAN* rs14035TT genotypes. HR, hazard ratio.

study of ischemic stroke, we focused on miRNA biogenesis genes, such as *DICER1*, *DROSHA*, *XPO5*, and *RAN*, which are related to endothelial miRNA expression and angiogenesis.[31,32] We evaluated six polymorphisms in *DICER1*, *DROSHA*, *XPO5*, and *RAN* genes essential for miRNA biosynthesis,[33-35] in ischemic stroke cases and controls. We found that polymorphisms in *DICER* and *DROSHA*, both of which are involved in angiogenesis and coagulation mechanisms,[36-38] were linked to ischemic stroke. Polymorphisms in *DICER*, a gene already known to play a role in vascular growth and genesis, displayed the strongest association with ischemic stroke. For example, the *DICER* rs3742330 GG genotype was significantly more frequent in both overall stroke cases and subtype SVD cases than in controls. Moreover, the interplay between the *DICER* rs3742330 GG genotype and hyperlipidemia status was elevated stroke prevalence. The roles of *DICER* in angiogenesis and vascular growth have previously been investigated, and there is a reported association between *DICER* expression and healthy endothelial cell growth.[39] Moreover, there is strong evidence that a functional DICER1-dependent pathway is essential for a healthy endothelial angiogenic response. All major steps of the angiogenic process, including adhesion, proliferation, migration, and capillary-like structure formation are compromised by disrupted DICER1 signaling in cerebromicrovascular endothelial cells,[40,41] in addition to other cell types.[32,38,42,43]

DICER and DROSHA play crucial roles in vertebrate development. DICER1-deficient mice die early in development, between embryonic days 12.5 and 14.5, displaying impaired blood vessel and yolk sac formation. Similarly, zebrafish *DICER* mutant embryos display abnormal morphogenesis during gastrulation, brain formation, somatogenesis, and heart development.[38] In addition, loss of DROSHA leads to vascular smooth muscle cells disorder followed by hypoplastic blood vessel walls, cardiomyopathy, and liver hemorrhage in mice between embryonic days 13.5 and

14.5, and causes embryonic mortality in affected mice.[44] A number of studies have reported that *DICER* and *DROSHA* polymorphisms, including rs3742330 and rs10719, affect disease development and patient survival in various cancers.[34,45-49] In addition, functional analysis of rs1057035, which resides in the 3'-UTR of *DICER*, has revealed that the polymorphism affects hsa-miR-574-3p targeting and DICER expression.[50] The *DROSHA* rs10719 polymorphism, which is located in the 3'-UTR of *DROSHA*, was associated with different *DROSHA* expression levels[33] and presented different binding efficiency for the target site of hsa-miR-27b.[35,51] Furthermore, previous studies reported that *DROSHA* rs6877842 and rs640831 polymorphisms affected miRNA expression levels.[34,52]

There are several potential mechanisms linking *DICER* and *DROSHA* polymorphisms to ischemic disease. First, *DICER* and *DROSHA* polymorphisms may directly affect angiogenesis via endothelial cell growth or induce blood vessel defects in embryos, resulting in vascular abnormalities.[39,44] In a DICER and DROSHA knockout model, hemorrhaging during vascular smooth muscle cell development was observed.[39,44] Moreover, DICER silencing in endothelial cells modulated the expression of several genes involved in endothelial biology, including nitric oxide synthase 3, matrix metalloproteinase 2 (MMP-2), integrins-v and -1, fibronectin, endothelin receptor types A, endothelin 1, vascular endothelial cadherin, and caspase-3. Both integrins-v and -1 are implicated in angiogenesis and endothelial survival,[53] and MMP-2 participates in autocrine processes that influence hypoxia-induced migration and apoptotic death in endothelial cells.[54] Additionally, DROSHA has a similar role to DICER in vascular smooth muscle cell survival through ERK1/2 and AKT regulation.[44] Furthermore, previous studies identified that DROSHA influenced the regulation of miRNA expression. Transcription of certain miRNAs does not require DICER, but does need DROSHA (e.g., miR-1225 and miR-228).[55] In addition, other miRNAs such as miR-877, miR-1224, and miR-1226 are independent of the canonical miRNA biogenesis pathway but dependent on the splicing process by *DROSHA*.[56-58]

Alternately, DICER and DROSHA may indirectly affect miRNA regulation via RNA interference. As noted above, *DICER* and *DROSHA* are involved in miRNA biogenesis. miRNAs have multiple mRNA targets, are important regulators of gene expression, and play important roles in the initiation and progression of diverse diseases including leukemia, rheumatoid arthritis, and multiple sclerosis.[59-62] In particular, miRNAs are known to affect the immune system and vasculature in ischemic stroke.[19,21,62] At present, it is not known whether *DICER* polymorphisms affect stroke risk by affecting DICER enzyme function or via RNA interference. Further *in vitro* studies are needed to distinguish between these two hypotheses.

Interestingly, the results of the current study indicate a significant association between increased mortality after stroke and the *RAN* rs14035 C>T polymorphism, after adjusting for age, sex, hypertension, hyperlipidemia, and smoking status. As we did not have data on causes of death, we cannot be certain that the high mortality in patients with *RAN* rs14035 TT genotype was due to vascular events. However, analysis revealed that the *RAN* rs14035 TT genotype was significantly associated with the survival rate of ischemic stroke patients, supporting the likelihood of it also causing post-stroke mortality.

This was a case-control association study with 988 samples, but it has several limitations. First, although we found an association between *XPO5* rs11077 polymorphisms and stroke risk, there is still no hypothesized mechanism for the role of this polymorphism in ischemic stroke prevalence. Second, the weak associations observed between the *DICER* rs13078, *DROSHA* rs6877842, and *RAN* rs14035 polymorphisms and ischemic stroke require replication. Third, some of the controls in our study were seeking medical attention; therefore, they were not completely healthy. However, recruitment of healthy participants with imaging and laboratory tests would markedly reduce the enrollment rate and including participants without imaging and laboratory tests may produce other vascular risk factor assessment biases. Finally, the study population was restricted to patients of Korean ethnicity.

Conclusions

We have identified an association between ischemic stroke susceptibility and polymorphisms in *DICER* rs3742330 and *DROSHA* rs10719, in addition to a significant association with the *RAN* rs14035 polymorphism in post-stroke mortality. These findings may encourage research efforts focusing on the role of *DICER* and *DROSHA* in vascular development. We postulate that the *DICER* rs3742330 and *DROSHA* rs10719 polymorphisms influence miRNA biosynthesis and therefore, miRNA post-transcriptional regulation during vascular endothelial cell growth, proliferation, and differentiation. However, the underlying mechanism remains to be elucidated in future research.

Supplementary materials

Supplementary materials related to this article can be found online at https://doi.org/10.5853/jos.2017.02586.

Disclosure

The authors have no financial conflicts of interest.

Acknowledgments

This study was partially supported by National Research Foundation of Korea Grants funded by the Korean Government (NRF-2015R1D1A1A09057432, NRF-2016R1D1A1B03930141, and NRF-2017R1D1A1B03029582) and by the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, Republic of Korea (grant number: HI16C1559).

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Supplementary Table 1. PCR-RFLP condition for microRNA machinery genes polymorphism

SNP	Ref. gene	Polymorphism	Forward primer (5′-3′)	Reverse primer (5′-3′)	Restriction enzyme*
rs13078	*DICER*	A>T	5′-CTA GTT TTC CTG CAG ACA ATG CA-3′	5′-GTA ATG CAC ATT CAC CAA AGT CA-3′	*Bcc* I
rs3742330		A>G	5′- GGT CTC AGT TTG GTG GCT TC -3′	5′- CCT GCC TTG ACA ACA TGA AA -3′	*Ban* II
rs10719	*DROSHA*	T>C	5′-CTA GTT TTC CTG CAG ACA ATG CA-3′	5′-GTA ATG CAC ATT CAC CAA AGT CA-3′	*Dra* III
rs6877842		G>C	5′-GGG CGC AAA AAC ATG AGT GAC-3′	5′-TCC TCT CCA CAG CAA CGG AAT A-3′	*Sau* 96I
rs14035	*RAN*	C>T	5′-GAA GCA CTT GCT CAA AAT CTG TGA C-3′	5′- TGC CAT CCA CTG ATG TTC CAT C-3′	*Bsl* I
rs11077	*XPO5*	A>C	5′-TGC TTT GGG CAA GAA TCT GGT CAC-3′	5′-TAA AGG GGA TGT TAG CAC TAA AGA AT -3′	*Bsm* I

PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; RAN, Ran GTPase; XPO5, exportin 5.
*All of the restriction enzymes were available from New England Biolabs (Ipswich, MA, USA) and the reaction conditions recommended by the instructions were used.

Supplementary Table 2. Comparison of genotype frequencies and AOR of *DICER* 6095 rs13078 A>T, *DICER* 9480 rs3742330 T>C, *DROSHA* -715 rs6877842 C>G, *DROSHA* 4576 rs10719 T>C, *RAN* 1857 rs14035 C>T, and *XPO5* 4485 rs11077 A>C polymorphisms between stroke subtype and controls

Genotype	Controls (n=403)	Single SVD (n=71)	AOR (95% CI)*	*P*	Multiple SVD (n=66)	AOR (95% CI)*	*P*
DICER rs13078 A>T							
AA	360 (89.3)	65 (91.5)	1.000 (reference)		55 (83.3)	1.000 (reference)	
AT	43 (10.7)	6 (8.5)	0.817 (0.321–2.081)	0.672	11 (16.7)	1.799 (0.845–3.830)	0.128
TT	0	0	NA		0	NA	
Dominant (AA vs. AT+TT)			0.817 (0.32–2.081)	0.672		1.799 (0.845–3.830)	0.128
Recessive (AA+AT vs. T)			NA			NA	
DICER rs3742330 T>C							
TT	148 (36.7)	20 (28.2)	1.000 (reference)		18 (27.3)	1.000 (reference)	
TC	180 (44.7)	33 (46.5)	1.380 (0.740–2.574)	0.312	37 (56.1)	1.763 (0.944–3.291)	0.075
CC	75 (18.6)	18 (25.4)	1.796 (0.855–3.775)	0.122	11 (16.7)	0.993 (0.424–2.328)	0.988
Dominant (TT vs. TC+CC)			1.490 (0.831–2.671)	0.181		1.549 (0.852–2.817)	0.152
Recessive (TT+TC vs. CC)			1.429 (0.769–2.658)	0.259		0.779 (0.380–1.600)	0.497
DROSHA rs6877842 C>G							
CC	371 (92.1)	67 (94.4)	1.000 (reference)		64 (97.0)	1.000 (reference)	
CG	31 (7.7)	4 (5.6)	0.777 (0.256–2.354)	0.655	1 (1.5)	0.189 (0.025–1.433)	0.107
GG	1 (0.2)	0	NA	0.998	1 (1.5)	8.288 (0.459–149.7)	0.152
Dominant (CC vs. CG+GG)			0.757 (0.250–2.288)	0.622		0.375 (0.086–1.639)	0.193
Recessive (CC+CG vs. GG)			NA	0.998		8.768 (0.489–157.1)	0.140
DROSHA rs10719 T>C							
TT	228 (56.6)	34 (47.9)	1.000 (reference)		37 (56.1)	1.000 (reference)	
TC	158 (39.2)	31 (43.7)	1.253 (0.722–2.174)	0.423	25 (37.9)	1.009 (0.572–1.782)	0.975
CC	17 (4.2)	6 (8.5)	1.837 (0.639–5.277)	0.259	4 (6.1)	1.690 (0.494–5.788)	0.403
Dominant (TT vs. TC+CC)			1.305 (0.768–2.215)	0.325		1.061 (0.616–1.828)	0.830
Recessive (TT+TC vs. CC)			1.596 (0.568–4.483)	0.375		1.583 (0.495–5.063)	0.439
RAN rs14035 C>T							
CC	240 (59.9)	43 (60.6)	1.000 (reference)		39 (59.1)	1.000 (reference)	
CT	149 (37.0)	25 (35.2)	0.860 (0.491–1.508)	0.600	25 (37.9)	0.930 (0.526–1.645)	0.803
TT	14 (3.5)	3 (4.2)	1.081 (0.257–4.541)	0.915	2 (3.0)	1.053 (0.222–4.998)	0.948
Dominant (CC vs. CT+TT)			0.879 (0.509–1.516)	0.642		0.939 (0.540–1.634)	0.824
Recessive (CC+CT vs. TT)			1.325 (0.342–5.145)	0.684		1.137 (0.244–5.296)	0.871
XPO5 rs11077 A>C							
AA	319 (79.2)	60 (84.5)	1.000 (reference)		57 (86.4)	1.000 (reference)	
AC	79 (19.6)	11 (15.5)	0.734 (0.358–1.503)	0.398	9 (13.6)	0.603 (0.278–1.307)	0.200
CC	5 (1.2)	0	NA	0.998	0	NA	0.998
Dominant (AA vs. AC+CC)			0.692 (0.339–1.414)	0.313		0.562 (0.260–1.215)	0.143
Recessive (AA+AC vs. CC)			NA	0.998		NA	0.998

Values are presented as number (%).
AOR, adjusted odds ratio; RAN, Ran GTPase; XPO5, exportin 5; LAD, large artery disease; SVD, small vessel disease; CI, confidence interval; NA, not available.
*The adjusted odds ratio on the basis of risk factors, such as age, gender, hypertension, diabetes mellitus, hyperlipidemia, smoking.

https://doi.org/10.5853/jos.2017.02586

Supplementary Table 3. AORs for ischemic stroke associated with microRNA machinery gene genotypes, stratified by clinical factors

Variable	*DICER1* rs13078 AA vs. AT+TT		*DICER1* rs3742330 AA vs. GG		*DROSHA* rs6877842 CC vs. CG+GG		*DROSHA* rs10719 TT vs. CC		*RAN* rs14035 CC vs. CT		*XPO5* rs11077 AA vs. AC+CC	
	AOR (95% CI)*	*P*	AOR (95% CI)*	*P*	AOR (95% CI)*	*P*	AOR (95% CI)*	*P*	AOR (95% CI)*	*P*	AOR (95% CI)*	*P*
Age (yr)												
<63	1.595 (0.783–3.251)	0.199	1.843 (1.039–3.267)	0.037	1.457 (0.676–3.140)	0.337	1.745 (0.748–4.072)	0.198	0.677 (0.447–1.025)	0.065	0.858 (0.517–1.424)	0.553
≥63	0.720 (0.407–1.273)	0.258	1.170 (0.703–1.946)	0.546	0.479 (0.231–0.991)	0.047	2.042 (0.838–4.978)	0.116	0.934 (0.633–1.379)	0.732	0.540 (0.333–0.877)	0.013
Sex												
Male	1.523 (0.764–3.034)	0.232	1.462 (0.795–2.691)	0.222	1.092 (0.462–2.581)	0.842	1.695 (0.632–4.546)	0.295	0.767 (0.494–1.191)	0.238	0.616 (0.365–1.040)	0.993
Female	0.759 (0.428–1.347)	0.347	1.511 (0.929–2.457)	0.096	0.720 (0.375–1.380)	0.322	2.040 (0.936–4.449)	0.073	0.831 (0.571–1.209)	0.333	0.707 (0.441–1.133)	0.149
Hypertension												
No	1.353 (0.728–2.516)	0.339	1.478 (0.853–2.560)	0.163	0.823 (0.386–1.756)	0.615	1.967 (0.840–4.607)	0.119	0.817 (0.543–1.228)	0.330	0.709 (0.431–1.165)	0.995
Yes	0.705 (0.387–1.284)	0.253	1.484 (0.880–2.502)	0.138	0.754 (0.377–1.510)	0.426	1.995 (0.830–4.796)	0.123	0.784 (0.529–1.162)	0.225	0.623 (0.384–1.009)	0.054
Diabetes mellitus												
No	1.061 (0.654–1.722)	0.811	1.557 (1.024–2.368)	0.039	0.840 (0.482–1.464)	0.539	1.770 (0.925–3.385)	0.085	0.770 (0.563–1.053)	0.102	0.700 (0.476–1.023)	0.065
Yes	0.696 (0.264–1.832)	0.463	1.247 (0.514–3.027)	0.625	0.534 (0.146–1.947)	0.342	3.509 (0.417–29.554)	0.248	0.941 (0.481–1.841)	0.860	0.540 (0.237–1.228)	0.141
Hyperlipidemia												
No	0.943 (0.579–1.536)	0.815	1.221 (0.785–1.897)	0.376	0.697 (0.374–1.298)	0.255	2.642 (1.301–5.363)	0.007	0.972 (0.699–1.352)	0.865	0.647 (0.428–0.978)	0.039
Yes	1.120 (0.417–3.006)	0.822	2.334 (1.074–5.075)	0.032	1.040 (0.407–2.655)	0.936	0.813 (0.238–2.780)	0.741	0.486 (0.278–0.849)	0.011	0.724 (0.382–1.376)	0.324
Smoker												
No	0.872 (0.500–1.521)	0.629	1.566 (0.987–2.485)	0.057	0.674 (0.362–1.254)	0.213	2.522 (1.196–5.323)	0.015	0.775 (0.546–1.101)	0.155	0.584 (0.371–0.919)	0.020
Yes	1.218 (0.595–2.494)	0.590	1.337 (0.681–2.628)	0.399	1.099 (0.430–2.805)	0.844	1.115 (0.382–3.254)	0.842	0.817 (0.504–1.326)	0.413	0.802 (0.462–1.390)	0.431
Folate†												
>3.55 nmol/L	1.160 (0.720–1.871)	0.542	1.578 (1.046–2.381)	0.030	0.772 (0.445–1.342)	0.359	2.051 (1.064–3.956)	0.032	0.802 (0.589–1.090)	0.159	0.663 (0.453–0.971)	0.035
≤3.55 nmol/L	0.816 (0.205–3.239)	0.772	0.751 (0.212–2.659)	0.657	0.681 (0.117–3.955)	0.669	1.999 (0.365–10.943)	0.425	1.210 (0.485–3.021)	0.683	0.551 (0.194–1.564)	0.263
Homocysteine‡												
<13.5 μmol/L	1.177 (0.721–1.919)	0.515	1.422 (0.938–2.155)	0.097	0.867 (0.503–1.496)	0.609	2.356 (1.245–4.457)	0.008	0.780 (0.574–1.061)	0.113	0.714 (0.489–1.044)	0.082
≥13.5 μmol/L	0.579 (0.194–1.723)	0.326	1.375 (0.501–3.773)	0.536	0.431 (0.090–2.077)	0.294	0.378 (0.042–3.424)	0.387	1.002 (0.466–2.155)	0.996	0.458 (0.186–1.128)	0.090

AOR, adjusted odds ratio; RAN, Ran GTPase; XPO5, exportin 5; CI, confidence interval.
*Odds ratios adjusted for age, sex, hypertension, diabetes mellitus, hyperlipidemia, and smoking status; †3.55 nmol/L corresponds to the lowest 15% of folate values in the sample; ‡13.5 μmol/L corresponds to the highest 15% of homocysteine values in the sample.

Supplementary Table 4. Adjusted odds ratios for ischemic stroke associated with *DICER* genotypes, combined by clinical factors

Characteristic	*DICER* rs13078 AA	*DICER* rs13078 AT+TT	*DICER* rs3742330 AA	*DICER* rs3742330 GG
Sex				
Male	1.000 (reference)	1.523 (0.764–3.034)	1.000 (reference)	1.462 (0.795–2.691)
Female	1.151 (0.808–1.639)	1.037 (0.542–1.986)	1.022 (0.562–1.859)	2.413 (1.208–4.819)
Age (yr)				
<63	1.000 (reference)	1.657 (0.812–3.384)	1.000 (reference)	1.843 (1.039–3.267)
≥63	0.876 (0.545–1.410)	0.813 (0.378–1.747)	1.081 (0.663–1.763)	1.381 (0.756–2.520)
Hypertension				
No	1.000 (reference)	1.353 (0.728–2.516)	1.000 (reference)	1.478 (0.853–2.560)
Yes	2.578 (1.933–3.439)	1.825 (0.986–3.379)	2.337 (1.452–3.760)	3.418 (2.028–5.762)
Diabetes mellitus				
No	1.000 (reference)	1.061 (0.654–1.722)	1.000 (reference)	1.557 (1.024–2.368)
Yes	2.230 (1.527–3.256)	1.524 (0.601–3.864)	2.133 (1.159–3.927)	2.033 (0.968–4.266)
Hyperlipidemia				
No	1.000 (reference)	0.943 (0.579–1.536)	1.000 (reference)	1.221 (0.785–1.897)
Yes	1.381 (1.003–1.901)	1.443 (0.556–3.751)	1.392 (0.826–2.345)	3.484 (1.740–6.977)
Smoker				
No	1.000 (reference)	0.872 (0.500–1.521)	1.000 (reference)	1.566 (0.987–2.485)
Yes	1.212 (0.853–1.721)	1.475 (0.720–3.021)	1.223 (0.693–2.158)	2.095 (1.042–4.213)
Folate (nmol/L)*				
>3.55	1.000 (reference)	1.160 (0.720–1.871)	1.000 (reference)	1.578 (1.046–2.381)
≤3.55	3.730 (2.307–6.029)	2.159 (0.617–7.553)	5.227 (2.347–11.64)	4.010 (1.587–10.13)
Homocysteine (μmol/L)†				
<13.5	1.000 (reference)	1.177 (0.721–1.919)	1.000 (reference)	1.422 (0.938–2.155)
≥13.5	1.904 (1.252–2.896)	1.067 (0.388–2.936)	1.845 (0.941–3.619)	2.365 (1.105–5.063)

*3.55 nmol/L corresponds to the lowest 15% of folate values in the sample; †13.5 μmol/L corresponds to the highest 15% of homocysteine values in sample.

https://doi.org/10.5853/jos.2017.02586

Supplementary Table 5. Adjusted odds ratios for ischemic stroke associated with *DROSHA* genotypes, combined by clinical factors

Characteristic	*DROSHA* rs6877842 CC	*DROSHA* rs6877842 CG+GG	*DROSHA* rs10719 TT	*DROSHA* rs10719 CC
Sex				
Male	1.000 (reference)	1.092 (0.462–2.581)	1.000 (reference)	1.695 (0.632–4.546)
Female	1.077 (0.761–1.523)	0.885 (0.431–1.820)	1.289 (0.834–1.992)	3.423 (1.390–8.427)
Age (yr)				
<63	1.000 (reference)	1.452 (0.674–3.130)	1.000 (reference)	1.708 (0.730–3.999)
≥63	0.811 (0.508–1.295)	0.581 (0.244–1.387)	0.670 (0.360–1.246)	2.046 (0.640–6.538)
Hypertension				
No	1.000 (reference)	0.823 (0.386–1.756)	1.000 (reference)	1.967 (0.840–4.607)
Yes	2.404 (1.815–3.184)	1.879 (0.924–3.823)	2.415 (1.666–3.501)	4.781 (1.981–11.54)
Diabetes mellitus				
No	1.000 (reference)	0.840 (0.482–1.464)	1.000 (reference)	1.770 (0.925–3.385)
Yes	2.193 (1.520–3.166)	1.104 (0.311–3.918)	2.351 (1.449–3.814)	12.046 (1.541–94.19)
Hyperlipidemia				
No	1.000 (reference)	0.697 (0.374–1.298)	1.000 (reference)	2.642 (1.301–5.363)
Yes	1.365 (0.994–1.874)	1.388 (0.563–3.421)	1.415 (0.947–2.114)	1.240 (0.401–3.832)
Smoker				
No	1.000 (reference)	0.674 (0.362–1.254)	1.000 (reference)	2.522 (1.195–5.323)
Yes	1.175 (0.836–1.652)	1.401 (0.545–3.602)	1.181 (0.766–1.819)	1.662 (0.583–4.739)
Folate (nmol/L)*				
>3.55	1.000 (reference)	0.772 (0.445–1.342)	1.000 (reference)	2.051 (1.064–3.956)
≤3.55	3.401 (2.144–5.397)	2.415 (0.435–13.40)	2.635 (1.488–4.665)	4.775 (0.984–23.17)
Homocysteine (μmol/L)†				
<13.5	1.000 (reference)	0.867 (0.503–1.496)	1.000 (reference)	2.356 (1.245–4.457)
≥13.5	1.798 (1.199–2.695)	0.830 (0.198–3.475)	2.020 (1.186–3.441)	1.289 (0.211–7.870)

*3.55 nmol/L corresponds to the lowest 15% of folate values in the sample; †13.5 μmol/L corresponds to the highest 15% of homocysteine values in sample.

Supplementary Table 6. Adjusted odds ratios for ischemic stroke associated with *RAN* and *XPO5* genotypes, combined by clinical factors

Characteristic	*RAN* rs14035 CC	*RAN* rs14035 CT	*XPO5* rs11077 AA	*XPO5* rs11077 AC+CC
Sex				
Male	1.000 (reference)	0.767 (0.494–1.191)	1.000 (reference)	0.616 (0.365–1.040)
Female	1.010 (0.656–1.557)	0.990 (0.588–1.666)	0.966 (0.668–1.397)	0.842 (0.473–1.498)
Age (yr)				
<63	1.000 (reference)	0.697 (0.460–1.057)	1.000 (reference)	0.861 (0.519–1.429)
≥63	0.634 (0.356–1.131)	0.703 (0.367–1.346)	0.761 (0.464–1.249)	0.686 (0.333–1.413)
Hypertension				
No	1.000 (reference)	0.817 (0.543–1.228)	1.000 (reference)	0.709 (0.431–1.165)
Yes	2.500 (1.758–3.556)	1.938 (1.297–2.894)	2.425 (1.795–3.277)	1.502 (0.918–2.457)
Diabetes mellitus				
No	1.000 (reference)	0.770 (0.563–1.053)	1.000 (reference)	0.698 (0.476–1.023)
Yes	1.918 (1.218–3.021)	1.877 (1.056–3.336)	2.223 (1.494–3.306)	1.229 (0.579–2.610)
Hyperlipidemia				
No	1.000 (reference)	0.972 (0.699–1.352)	1.000 (reference)	0.647 (0.428–0.978)
Yes	1.837 (1.227–2.749)	0.934 (0.593–1.472)	1.403 (0.996–1.977)	1.027 (0.575–1.836)
Smoker				
No	1.000 (reference)	0.775 (0.546–1.101)	1.000 (reference)	0.584 (0.371–0.919)
Yes	1.208 (0.790–1.847)	1.207 (0.727–2.002)	1.133 (0.783–1.637)	1.079 (0.621–1.877)
Folate (nmol/L)*				
>3.55	1.000 (reference)	0.802 (0.590–1.090)	1.000 (reference)	0.663 (0.453–0.971)
≤3.55	3.156 (1.791–5.564)	2.833 (1.360–5.900)	3.457 (2.075–5.758)	2.155 (0.867–5.355)
Homocysteine (μmol/L)†				
<13.5	1.000 (reference)	0.780 (0.574–1.061)	1.000 (reference)	0.714 (0.489–1.044)
≥13.5	1.450 (0.882–2.383)	1.566 (0.827–2.969)	1.865 (1.197–2.907)	0.872 (0.392–1.939)

RAN, Ran GTPase; XPO5, exportin 5.
*3.55 nmol/L corresponds to the lowest 15% of folate values in the sample; †13.5 μmol/L corresponds to the highest 15% of homocysteine values in sample.

https://doi.org/10.5853/jos.2017.02586

Supplementary Table 7. Altered blood coagulation factors according to miRNA processing gene genotypes

Genotype	PLT		PT (sec)		aPTT (sec)		Fibrinogen		Antithrombin	
	Mean±SD (n)	CV (%)	Mean±SD (n)	CV (%)	Mean±SD (n)	CV (%)	Mean±SD (n)	CV (%)	Mean±SD (n)	CV (%)
DICER1 rs13078 A>T										
AA	246.77±81.74 (879)	33.1	11.79±0.94 (765)	8.0	31.52±11.89 (765)	37.7	418.21±129.43 (601)	30.9	93.46±18.55 (605)	19.8
AT	247.18±63.58 (97)	25.7	11.67±0.77 (85)	6.6	31.1±6.63 (85)	21.3	439.63±120.52 (65)	27.4	93.85±18.42 (65)	19.6
TT	214.5±14.85 (2)	6.9	11.3±0.57 (2)	5.0	27.35±3.32 (2)	12.1	406.50±78.49 (2)	19.3	78.00±9.9 (2)	12.7
P	0.849		0.385		0.835		0.433		0.492	
DICER1 rs3742330 A>G										
AA	246.99±92.07 (315)	37.3	11.85±1.12 (274)	9.5	31.13±6.16 (276)	19.8	410.30±120.95 (210)	29.5	93.08±15.17 (212)	16.3
AG	247.58±69.72 (454)	28.2	11.76±0.79 (396)	6.7	31.06±6.99 (395)	22.5	427.96±133.83 (309)	31.3	94.07±19.11 (310)	20.3
GG	244.58±81.77 (209)	33.4	11.73±0.86 (182)	7.3	32.87±21.3 (181)	64.8	418.45±127.35 (149)	30.4	92.67±21.41 (150)	23.1
P	0.903		0.308		0.178		0.299		0.708	
DROSHA rs6877842 C>G										
CC	246.18±79.98 (909)	32.5	11.78±0.92 (796)	7.8	31.39±11.21 (796)	35.7	419.72±130.14 (623)	31.0	93.49±18.42 (628)	19.7
CG	253.02±79.67 (67)	31.5	11.78±0.93 (55)	7.9	32.55±14.76 (55)	45.3	429.26±102.89 (44)	24.0	92.67±20.19 (43)	21.8
GG	295.5±144.96 (2)	49.1	11.5±0 (1)	0.0	35.4±0 (1)	0.0	406 (1)		105 (1)	
P	0.549		0.954		0.722		0.894		0.790	
DROSHA rs10719 T>C										
TT	246.71±75.2 (528)	30.5	11.75±0.79 (454)	6.7	31.07±7.06 (456)	22.7	419.98±160.20 (358)	38.1	94.67±17.64 (367)	18.6
TC	249.64±86.03 (387)	34.5	11.79±1.06 (341)	9.0	31.2±7.3 (339)	23.4	417.08±125.99 (262)	30.2	91.02±17.50 (257)	19.2
CC	229.29±80.02 (63)	34.9	11.94±1 (57)	8.4	36.23±35.28 (57)	97.4	439.99±160.20 (48)	36.4	97.32±27.29 (48)	28.0
P	0.173		0.352		0.005		0.525		0.017	
RAN 1rs14035 C>T										
CC	246.41±83.11 (603)	33.7	11.76±0.8 (531)	6.8	31.02±7.31 (531)	23.6	420.57±126.71 (419)	30.1	93.72±19.24 (421)	20.5
CT	246.1±75.26 (338)	30.6	11.78±1.07 (287)	9.1	32.49±16.96 (287)	52.2	419.74±134.66 (221)	32.1	92.03±15.98 (223)	17.4
TT	258.03±71.59 (37)	27.7	12.04±1.37 (34)	11.4	29.75±4.54 (34)	15.3	420.44±107.37 (28)	25.5	100.62±24.28 (28)	24.1
P	0.682		0.252		0.146		0.997		0.061	
XPO5 rs11077 A>C										
AA	247.43±82.24 (810)	33.2	11.79±0.95 (708)	8.1	31.65±12.4 (708)	39.2	417.55±127.20 (561)	30.5	93.17±17.48 (562)	18.8
AC	244.47±68.65 (162)	28.1	11.7±0.76 (138)	6.5	30.42±4.51 (138)	14.8	433.61±132.90 (103)	30.6	95.03±23.41 (106)	24.6
CC	216.5±61.75 (6)	28.5	12.42±0.84 (6)	6.8	33.68±5.49 (6)	16.3	453.99±201.62 (4)	44.4	90.36±19.84 (4)	22.0
P	0.593		0.145		0.461		0.437		0.609	

P-value calculated by one-way analysis of variance test.
PLT, platelet; PT, prothrombin time; aPTT, activated partial thromboplastin time; CV, coefficient of variation; RAN, Ran GTPase; XPO5, exportin 5.

Supplementary Table 8. Genotype frequencies of microRNA processing genes polymorphism and ischemic stroke patients' mortality according to TOAST subgroup

Genotype	Validation set		Adjusted HR (95% CI)*	*P*	Validation set		Adjusted HR (95% CI)*	*P*	Validation set		Adjusted HR (95% CI)*	*P*
	Stroke LAD (n=200)	Death (n=32)			Stroke SVD (n=149)	Death (n=14)			Stroke CE (n=54)	Death (n=15)		
DICER1 rs13078 A>T												
AA	186 (93.0)	29 (90.6)	1.000 (reference)		130 (87.2)	12 (85.7)	1.000 (reference)		49 (90.7)	15 (100.0)	1.000 (reference)	
AT	14 (7.0)	3 (9.4)	1.337 (0.399–4.477)	0.638	19 (12.8)	2 (14.3)	1.466 (0.310–6.923)	0.629	4 (7.4)	0	NA	0.978
TT	0	0			0	0			1 (1.9)	0		
Dominant (AA vs. AT+TT)			1.337 (0.399–4.477)	0.638			1.466 (0.310–6.923)	0.629			NA	0.978
Recessive (AA+AT vs. TT)			NA				NA				NA	
DICER1 rs3742330 A>G												
AA	62 (31.0)	9 (28.1)	1.000 (reference)		40 (26.8)	3 (21.4)	1.000 (reference)		18 (33.3)	5 (33.3)	1.000 (reference)	
AG	89 (44.5)	18 (56.3)	1.612 (0.706–3.678)	0.257	78 (52.3)	8 (57.1)	1.527 (0.382–6.103)	0.549	20 (37.0)	3 (20.0)	0.687 (0.112–4.236)	0.686
GG	49 (24.5)	5 (15.6)	0.496 (0.149–1.645)	0.252	31 (20.8)	3 (21.4)	1.078 (0.201–5.775)	0.930	16 (29.6)	7 (46.7)	2.050 (0.583–7.214)	0.263
Dominant (AA vs. AG+GG)			1.217 (0.552–2.681)	0.627			1.414 (0.387–5.161)	0.600			1.649 (0.511–5.322)	0.403
Recessive (AA+AG vs. GG)			0.508 (0.193–1.334)	0.169			1.017 (0.275–3.764)	0.980			2.346 (0.760–7.246)	0.138
DROSHA rs6877842 C>G												
CC	188 (94.0)	32 (100.0)	1.000 (reference)		143 (96.0)	14 (100.0)	1.000 (reference)		48 (88.9)	13 (86.7)	1.000 (reference)	
CG	12 (6.0)	0	NA	0.954	5 (3.4)	0	NA	0.954	6 (11.1)	2 (13.3)	1.046 (0.232–4.723)	0.953
GG	0	0			1 (0.7)	0	NA	0.985	0	0		
Dominant (CC vs. CG+GG)			NA	0.954			NA	0.987			1.046 (0.232–4.723)	0.953
Recessive (CC+CG vs. GG)							NA	0.987				
DROSHA rs10719 T>C												
TT	106 (53.0)	17 (53.1)	1.000 (reference)		78 (52.3)	8 (57.1)	1.000 (reference)		29 (53.7)	7 (46.7)	1.000 (reference)	
TC	82 (41.0)	12 (37.5)	0.867 (0.412–1.826)	0.708	60 (40.3)	5 (35.7)	0.868 (0.266–2.835)	0.815	18 (33.3)	4 (26.7)	2.351 (0.529–10.439)	0.261
CC	12 (6.0)	3 (9.4)	1.407 (0.393–5.034)	0.600	11 (7.4)	1 (7.1)	1.432 (0.160–12.800)	0.748	7 (13.0)	4 (26.7)	3.557 (0.859–14.735)	0.080
Dominant (TT vs. TC+CC)			0.916 (0.455–1.845)	0.806			0.906 (0.290–2.831)	0.866			2.165 (0.719–6.514)	0.170
Recessive (TT+TC vs. CC)			1.297 (0.387–4.349)	0.674			1.161 (0.144–9.367)	0.888			2.501 (0.718–8.714)	0.150
RAN rs14035 C>T												
CC	119 (59.5)	16 (50.0)	1.000 (reference)		89 (59.7)	7 (50.0)	1.000 (reference)		39 (72.2)	13 (86.7)	1.000 (reference)	
CT	74 (37.0)	13 (40.6)	1.182 (0.558–2.504)	0.663	54 (36.2)	5 (35.7)	1.233 (0.370–4.107)	0.733	13 (24.1)	2 (13.3)	1.027 (0.174–6.057)	0.977
TT	7 (3.5)	3 (9.4)	5.978 (1.422–25.139)	0.015	6 (4.0)	2 (14.3)	9.403 (1.542–57.337)	0.015	2 (3.7)	0	NA	0.985
Dominant (CC vs. CT+TT)			1.389 (0.684–2.820)	0.363			1.712 (0.571–5.134)	0.337			0.891 (0.159–4.981)	0.895
Recessive (CC+CT vs. TT)			3.976 (1.111–14.235)	0.034			5.223 (1.090–25.038)	0.039			NA	0.987

https://doi.org/10.5853/jos.2017.02586

Supplementary Table 8. Continued

Genotype	Validation set		Adjusted HR (95% CI)*	*P*	Validation set		Adjusted HR (95% CI)*	*P*	Validation set		Adjusted HR (95% CI)*	*P*
	Stroke LAD (n=200)	Death (n=32)			Stroke SVD (n=149)	Death (n=14)			Stroke CE (n=54)	Death (n=15)		
XPO5 rs11077 A>C												
AA	171 (85.5)	28 (87.5)	1.000 (reference)		125 (83.9)	11 (78.6)	1.000 (reference)		48 (88.9)	13 (86.7)	1.000 (reference)	
AC	29 (14.5)	4 (12.5)	0.961 (0.324–2.848)	0.943	24 (16.1)	3 (21.4)	0.878 (0.224–3.438)	0.852	6 (11.1)	2 (13.3)	2.162 (0.357–13.092)	0.402
CC	0	0			0	0			0	0		
Dominant (AA vs. AC+CC)			0.961 (0.324–2.848)	0.943			0.878 (0.224–3.438)	0.852			2.162 (0.357–13.092)	0.402
Recessive (AA+AC vs. CC)			NA				NA				NA	

Values are presented as number (%).
TOAST, Trial of Org 10172 in Acute Stroke Treatment; LAD, large artery disease; HR, hazard ratio; CI, confidence interval; SVD, small vessel disease; CE, cardioembolism; NA, not available; RAN, Ran GTPase; XPO5, exportin 5.
*Adjusted for age, sex, hypertension, diabetes mellitus, hyperlipidemia, and smoking status based on cox-regression analysis.

Supplementary Table 9. Results of stepwise Cox regression analysis of ischemic stroke survival

Covariate	β	SEM	HR (95% CI)	*P**
RAN rs14035 CC vs. TT in LAD group				
Age	0.083	0.025	1.086 (1.034–1.141)	0.001
RAN rs14035 CC+CT vs. TT in LAD group				
Age	0.051	0.018	1.052 (1.016–1.090)	0.005
RAN rs14035 CC vs. TT in SVD group				
Genetic variant (CC vs. TT)	1.960	0.851	7.100 (1.340–37.629)	0.021
Age	0.098	0.033	1.103 (1.035–1.176)	0.003
RAN rs14035 CC+CT vs. TT in SVD group				
Genetic variant (CC+CT vs. TT)	1.573	0.769	4.819 (1.067–21.764)	0.041
Age	0.057	0.024	1.058 (1.010–1.110)	0.020

SEM, standard error of the mean; HR, hazard ratio; CI, confidence interval; RAN, Ran GTPase; LAD, large artery disease; SVD, small vessel disease.
**P*-value calculated by Cox proportional-hazards regression based on stepwise method.

https://doi.org/10.5853/jos.2017.02586

Supplementary Table 10. Frequency of *DICER* and *DROSHA* genotype combinations predicted by multidimensional reduction in ischemic stroke cases and controls

Genotype	Controls (n=403)	Case (n=585)	AOR (95% CI)*	$P^{\dagger}$	$P^{\ddagger}$
DICER rs13078 A>T/*DICER* rs3742330 A>G					
AA/AA	123 (30.5)	152 (26.0)	1.000 (reference)		
AA/AG	165 (40.9)	243 (41.5)	1.139 (0.821–1.580)	0.435	0.435
AA/GG	70 (17.4)	132 (22.6)	1.351 (0.911–2.002)	0.134	0.179
AA/AG+GG	237 (58.8)	375 (64.1)	1.209 (0.892–1.639)	0.220	0.251
AT/AA	25 (6.2)	17 (2.9)	0.546 (0.273–1.092)	0.087	0.139
AT/AG	15 (3.7)	34 (5.8)	1.881 (0.958–3.695)	0.067	0.139
AT/AG+GG	18 (4.5)	38 (6.5)	1.748 (0.930–3.285)	0.083	0.139
AT+TT/AA	25 (6.2)	17 (2.9)	0.546 (0.273–1.092)	0.087	0.139
AT+TT/AG	15 (3.7)	37 (6.3)	2.033 (1.044–3.961)	0.037	0.139
DROSHA rs6877842 C>T/*DROSHA* rs10719 T>C					
CC/TT	206 (51.1)	281 (48.0)	1.000 (reference)		
CC/TC	148 (36.7)	221 (37.8)	1.083 (0.812–1.445)	0.587	0.587
CC/CC	17 (4.2)	46 (7.9)	2.005 (1.093–3.678)	0.025	0.125
CC/TC+CC	165 (40.9)	267 (45.6)	1.177 (0.893–1.553)	0.248	0.587
CG/TT	21 (5.2)	22 (3.8)	0.788 (0.410–1.514)	0.474	0.587
CG+GG/TT	22 (5.5)	23 (3.9)	0.787 (0.415–1.491)	0.462	0.587

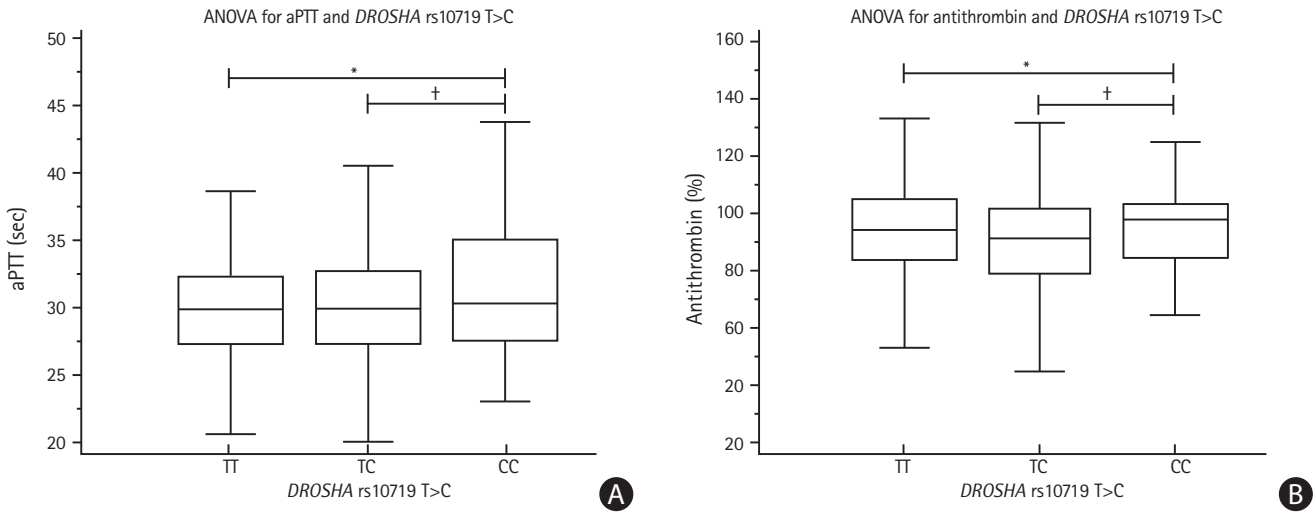
Values are presented as number (%). Combinations with frequencies of less than 5% in cases and controls are not shown.
AOR, adjusted odds ratios; CI, confidence interval.
*Odds ratio adjusted for age, sex, hypertension, diabetes mellitus, hyperlipidemia, and smoking status; †*P*-value calculated by logistics regression analysis; ‡*P*-value calculated by false discovery rate test.

Supplementary Table 11. Allele combinations of *DICER*, *DROSHA*, *RAN*, and *XPO5* polymorphisms between ischemic stroke patients and control subjects by multidimensional reduction method

Haplotype	Controls (2n=806)	Cases (2n=1,170)	OR (95% CI)*	*P*†	*P*‡
DICER rs13078 A>T-*DICER* rs3742330 A>G-*DROSHA* rs6877842 C>G-*DROSHA* rs10719 T>C-*RAN* rs14035 C>T-*XPO5* rs11077 A>C					
A-A-C-T-C-A	207 (25.7)	301 (25.7)	1.000 (reference)		
A-A-C-T-C-C	32 (4.0)	23 (2.0)	0.645 (0.368–1.129)	0.157	0.165
A-A-C-T-T-C	9 (1.1)	3 (0.3)	0.299 (0.080–1.116)	0.079	0.095
A-A-C-C-C-C	9 (1.1)	3 (0.3)	0.299 (0.080–1.116)	0.079	0.095
A-A-C-C-T-C	0	14 (1.2)	26.02 (1.544–438.5)	0.0002	0.001
A-A-G-T-C-A	8 (1.0)	0	0.053 (0.003–0.919)	0.003	0.009
A-G-C-T-C-A	184 (22.8)	286 (24.4)	1.394 (1.088–1.786)	0.010	0.024
A-G-C-T-C-C	21 (2.6)	14 (1.2)	0.598 (0.298–1.200)	0.165	0.165
A-G-C-T-T-A	36 (4.5)	70 (6.0)	1.744 (1.130–2.693)	0.014	0.028
A-G-C-C-C-A	39 (4.8)	86 (7.4)	1.978 (1.309–2.988)	0.001	0.004
A-G-C-C-C-C	1 (0.1)	18 (1.5)	16.15 (2.140–121.8)	0.0002	0.001
A-G-G-T-C-A	6 (0.7)	19 (1.6)	2.841 (1.118–7.218)	0.024	0.038
T-G-C-T-C-A	2 (0.2)	11 (0.9)	4.934 (1.083–22.47)	0.025	0.038
DICER rs3742330 A>G-*DROSHA* rs6877842 C>G-*DROSHA* rs10719 T>C-*RAN* rs14035 C>T-*XPO5* rs11077 A>C					
A-C-T-C-A	220 (27.3)	321 (27.4)	1.000 (reference)		
A-C-T-C-C	34 (4.2)	23 (2.0)	0.464 (0.266–0.809)	0.007	0.011
A-C-T-T-A	74 (9.2)	70 (6.0)	0.648 (0.448–0.938)	0.023	0.031
A-C-T-T-C	11 (1.4)	3 (0.3)	0.187 (0.052–0.678)	0.006	0.011
A-C-C-T-C	0	14 (1.2)	19.89 (1.180–335.4)	0.001	0.003
A-G-T-C-A	8 (1.0)	0	0.040 (0.002–0.703)	0.001	0.003
G-C-T-C-C	22 (2.7)	15 (1.3)	0.467 (0.237–0.921)	0.037	0.042
G-C-C-C-A	39 (4.8)	88 (7.5)	1.546 (1.022–2.340)	0.043	0.043
G-C-C-C-C	1 (0.1)	20 (1.7)	13.71 (1.825–102.9)	0.0004	0.003
DICER rs3742330 A>G-*DROSHA* rs10719 T>C-*RAN* rs14035 C>T-*XPO5* rs11077 A>C					
A-T-C-A	230 (28.5)	323 (27.6)	1.000 (reference)		
A-T-C-C	35 (4.3)	25 (2.1)	0.509 (0.296-0.873)	0.014	0.024
A-T-T-A	77 (9.6)	79 (6.8)	0.731 (0.511–1.044)	0.099	0.099
A-T-T-C	11 (1.4)	4 (0.3)	0.259 (0.081–0.824)	0.017	0.024
A-C-C-C	12 (1.5)	4 (0.3)	0.237 (0.076–0.746)	0.010	0.023
A-C-T-C	0	14 (1.2)	20.66 (1.226–348.4)	0.001	0.004
G-T-C-C	26 (3.2)	17 (1.5)	0.466 (0.247–0.878)	0.024	0.028
G-C-C-C	0	20 (1.7)	29.21 (1.757–485.8)	<0.0001	0.0001
DICER rs3742330 A>G-*DROSHA* rs10719 T>C-*XPO5* rs11077 A>C					
A-T-A	308 (38.2)	403 (34.4)	1.000 (reference)		
A-T-C	44 (5.5)	28 (2.4)	0.486 (0.296–0.799)	0.004	0.010
G-T-A	233 (28.9)	390 (33.3)	1.279 (1.027–1.594)	0.029	0.048
G-T-C	28 (3.5)	22 (1.9)	0.601 (0.337–1.070)	0.104	0.104
G-C-A	65 (8.1)	121 (10.3)	1.423 (1.017–1.991)	0.045	0.056
G-C-C	3 (0.4)	20 (1.7)	5.095 (1.500–17.31)	0.004	0.010
DICER rs3742330 A>G-*XPO5* rs11077 A>C					
A-A	418 (51.9)	570 (48.7)	1.000 (reference)		
A-C	57 (7.1)	48 (4.1)	0.618 (0.412–0.925)	0.022	0.022
G-A	298 (37.0)	511 (43.7)	1.257 (1.039–1.522)	0.020	0.022

Values are presented as number (%).
RAN, Ran GTPase; XPO5, exportin 5; OR, odds ratio; CI, confidence interval.
*Odds ratios was calculated to reference for total frequency; †*P*-value calculated by chi-square test; ‡*P*-value calculated by false discovery rate test.

https://doi.org/10.5853/jos.2017.02586



Supplementary Figure 1. Differences in activated partial thromboplastin time (aPTT) and antithrombin proportions based on *DROSHA* rs10719 T>C in ischemic stroke patients. Statistical analysis was performed using analysis of variance (ANOVA) test or Student t-test for each *DROSHA* rs10719 T>C genotype. (A) aPTT: the blood coagulation time was significantly different (P=0.005) between the *DROSHA* rs10719 TT (31.07±7.06), TC (31.20±7.30), and CC (36.23±35.28) genotypes. (B) Plasma antithrombin proportion: it was found that the *DROSHA* rs10719 T>C polymorphism affected the antithrombin proportion. The *DROSHA* rs10719CC genotype was associated with an elevated antithrombin percentage (97.32±27.29) compared with the *DROSHA* rs10719TT genotype (94.67±17.64), which had high antithrombin proportion relative to the *DROSHA* rs10719CC genotype (P=0.017). *P<0.05 calculated by ANOVA test; †P<0.05 calculated by Student t-test.