Genetic polymorphisms of pri-let-7f, gene–environment and gene–gene interactions, and associations with ischemic stroke risk in Liaoning Province

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Abstract

Objective: The incidence of stroke has been rising annually and investigations into traditional risk factors have led to increased attention on genetic factors. In this study, we focused on the pri-let-7f gene, and investigated the association between pri-let-7f gene polymorphisms and ischemic stroke (IS).

Methods: This case–control study included 1803 patients and 1456 healthy controls of Han ethnicity living in Liaoning Province. We carried out genotyping analysis of two loci, pri-let-7f-1 rs10739971 and pri-let-7f-2 rs17276588, and performed statistical analysis controlling for confounding factors by logistic regression.

Results: The A alleles and AA genotypes of both loci were significantly associated with an increased risk of IS. Variant genotypes of rs17276588 may also increase the risk of IS in females with alcohol intake. Gene–gene interaction analysis showed combined effects of mutations in both these single nucleotide polymorphisms (SNPs).

Conclusions: This study demonstrated an association between pri-let-7f SNPs and IS, providing potential latent biomarkers for the risk of IS. However, more detailed studies are needed to clarify these results.

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Introduction

Stroke is a leading cause of death globally and has a significant impact on human health, with high morbidity and an annual mortality of 5.5 million.[1] Almost 50% of stroke patients have to live with a chronic handicap for the rest of their life, and cerebrovascular disease is the second-leading cause of disability-adjusted life years.[2] China has the highest lifetime risk of stroke, estimated as 39.9%.[3] The prevalence of stroke in China has increased annually from 2013 to 2019, especially in northeast China,[4] and the high disability rate and high lifetime risk, together with the high prevalence, place a financial burden on society and physical and psychological burdens on caregivers. Stroke can be classified as ischemic stroke (IS) or hemorrhagic stroke, with IS accounting for around 80% of all stroke cases.[5] There is thus an urgent need to identify the mechanism underlying IS, to improve stroke-prevention strategies, treatment methods, and prognosis. Traditional risk factors for IS, such as age, sex, and hypertension, fail to explain stroke risk adequately[6]; however, genetic variables have been shown to participate in the pathophysiology of neurological diseases,[7,8] and increasing evidence suggests that genes may play a potential role in the pathophysiology of IS.

MicroRNAs (miRNAs) are endogenous non-coding RNAs that regulate mRNAs to affect their translation.[9,10] MiRNAs are initially transcribed as hairpin-containing primary transcripts (pri-miRNAs) by RNA polymerase II and then processed into precursor miRNAs (pre-miRNAs).[11]

Previous studies have demonstrated the functional influence of pri-miRNAs on miRNAs; e.g., methyltransferase 1-mediated methylation regulated the structure of let-7 by changing the structure within the pri-miRNA.[12] Let-7, the first known human miRNA, is related to stem cell division and differentiation. Its family consists of 10 members, designated a–j respectively, derived from 13 precursor sequences. Let-7 miRNAs have highly conserved sequences and functions.[13] Single nucleotide polymorphisms (SNPs) have been suggested to act as risk factors in some diseases,[14] and the association between SNPs and IS has been explored using genome-wide association studies and candidate-gene studies.[15–19] However, these explorations are complicated by the need for accurate selection and verification from among large numbers of potential SNPs, as well as the complex pathogenesis of IS and the difficulty in avoiding racial factors. Nevertheless, it is important to continue such studies, with suitable precautions, especially in light of developing methodologies.

Researchers found that pri-let-7 SNPs may affect the expression of mature let-7, and numerous studies have investigated the relationship between pri-let-7 SNPs and cancers.[20,21] Let-7f exerts functions in cerebral ischemia by targeting *NDRG3*, indicating its role in IS.[22] Two SNPs in the primary precursor area of the let-7f family (pri-let-7f-1 rs10739971, pri-let-7f-2 rs17276588) have been associated with cancers,[20,23] confirming the relevance of pri-let-7f and let-7f; however, information on

the association between these pri-let-7f SNPs and IS is still lacking.

In this study, we conducted a case–control study of 3259 subjects from northeast China, including 1803 patients with IS and 1456 healthy controls. We analyzed the two chosen SNPs, pri-let-7f-1 rs10739971 and pri-let-7f-2 rs17276588, using dominant and recessive models. The locus rs17276588 is located on the X chromosome, resulting in a sex difference. We determined the additive and multiplicative effects and explored the gene–gene and gene–environment interactions using logistic regression. We aimed to clarify the latent link between these two SNPs and IS, to further our understanding of primary IS.

Materials and methods

Subjects and design

This case–control study included patients with IS and age- and sex-matched healthy controls. Patient data were collected from the First Affiliated Hospital of China Medical University from December 2013 to December 2018. Eligible patients were first diagnosed with acute IS according to the following criteria: (1) abrupt onset of focused neurological deficits; (2) deficits that lasted longer than 24 hours; and (3) available brain imaging of the infarction. Patients with transient ischemic attack, cardioembolism, brain trauma, cerebrovascular malformations, coagulation dysfunction, autoimmune disorders, malignancies, chronic infectious diseases, or diseases of other systems were excluded from the study. Controls were recruited from individuals who underwent physical examination at the First Affiliated Hospital of China Medical University, with no evidence of stroke or other neurological illnesses.[17–19,24] Body mass index (BMI) was classified in accordance with the Asian obesity classification, and a BMI >22.9 was considered overweight. Hyperlipidemia was defined according to current criteria (triglycerides >1.7 mmol/L or/and total cholesterol >5.72 mmol/L).

This study was authorized by the Institutional Ethics Committee of China Medical University First Hospital on 20 February 2012 (No. 2012-38-1), and performed according to the World Medical Association's Code of Ethics (Declaration of Helsinki). All the participants provided written informed consent and their details were de-identified for privacy. The protocol was recorded in the Chinese Clinical Trial Registry (registration number: ChiCTR-COC-17013559) on 27 December 2017. The reporting of this study conforms to the Genetic Risk Prediction Studies (GRIPS) guidelines.[25]

SNP selection

SNPs were searched using the UCSC genome browser (http://genome.ucsc.edu/) and dbSNP database (https://www.ncbi.nlm.nih.gov/snp/?term = dbSNP). We identified two SNPs in the primary precursor region of let-7f (pri-let-7f-1 rs10739971, pri-let-7f-2 rs17276588) with a minor allele frequency (MAF). SNPs with a MAF >5% in the Chinese population were all tagSNPs, but their potential predicting roles were unclear. We selected these two SNPs on the basis of the following criteria: (1) located in the region −1 kb upstream of pri-let-7f; and (2) MAF >0.1 in Han Chinese. The two SNPs were rs10739971, located in the −949 base pairs upstream of pri-let-7f-1, and rs17276588 located in the −183 base pairs upstream of pri-let-7f-2 (Table 1).

DNA extraction and genotyping

Genomic DNA was extracted from ethylenediaminetetraacetic acid-anticoagulated peripheral blood and stored at −80°C. The SNaPshot reaction was performed, as

Table 1. Characteristics of the let-7f single nucleotide polymorphisms selected for study.

SNP ID	Gene	Chromosome	HWE p value	Genomic location	Alleles (major/minor)	MAF
rs10739971	pri-let-7f-1	9	0.299	96937680	G/A	0.389
rs17276588	pri-let-7f-2	X	0.952	53601143	G/A	0.274

SNP, single nucleotide polymorphism; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency.

Table 2. Primer sequences.

SNP	Primer sequence	Product size (bp)
rs10739971	Forward: 5′-TGGACTCTGCCTTCAATCCACAT-3′ Reverse: 5′-CATCATGCATAATCCAAATGCACTAAC-3′	221
rs17276588	Forward: 5′-TCAGCCTATGTGGGCCAGCTAC-3′ Reverse: 5′-GATGGTCTGGGTGGAGGATGGT-3′	227

SNP, single nucleotide polymorphism; bp, base pairs.

described previously.[17,18] DNA was extracted using a DNA Purification Kit (Promega, Madison, WI, USA) and genotype analysis was carried out using a SNaPshot Multiplex Kit (Applied Biosystems Co., Ltd., Foster City, CA, USA). The primer sequences used for polymerase chain reaction are shown in Table 2. The results were analyzed using an ABI 3130XL DNA sequence detector and GeneMapper 4.0 (Applied Biosystems Co., Ltd.).

Statistical analyses

All statistical analyses were performed using SPSS v23.0 (IBM Corp., Armonk, NY, USA), unless otherwise noted. All tests were two-tailed and significance was defined as $p < 0.05$. We compared the distributions of demographic variables using Pearson's χ^2 test and examined differences between risk factors and genotypes for alleles between patients and controls. A goodness-of-fit χ^2 test was used to test the Hardy–Weinberg equilibrium for each genotype. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression to estimate the association between IS and a particular genotype. The QUANTO power calculator was used to calculate the power in a cohort. Assuming a genotypic relative risk for the dominant model of 2, a MAF of 0.3, a 1.12% population prevalence of IS, and a Type I error probability of 0.05 in a sample size of 1803 patient samples and 1456 healthy controls, we would be able to reject the null hypothesis that $OR = 1$ with a power of 99.99% with a Type II error probability of 0.0001.

The attributable proportion due to interaction (AP) and relative excess risk due to interaction (RERI) were used to test additive gene–gene and gene–environment interactions. If RERI and $AP = 0$, there was no biologic interaction.[26] Gene interactions between the two SNPs were evaluated under additive and multiplicative models, using a calculator in Excel (available at http://www.epinet.se).[27] Confounding factors including sex, age, BMI, hypertension, diabetes mellitus, history of alcohol use, history of smoking, family history, and hyperlipidemia were controlled in the logistic regression analysis.

Results

Characteristics of study subjects

The study enrolled 1803 patients with IS and 1456 age - and sex-matched healthy controls, all of Han ethnicity, living in Liaoning Province in northern China. All patients were aged 40 to 80 years old. The study flow chart is shown in Figure 1 and the essential characteristics of the selected cases and controls, as well as the risk factors of IS, are summarized in Table 3. There was no significant difference between the cases and controls in terms of age or sex, but BMI, diabetes mellitus, hypertension, smoking history, family history, history of alcohol use, and hyperlipidemia all differed significantly between the two groups ($p < 0.001$), in accordance with conventional risk factors. Patients tended to have higher total cholesterol, triglyceride, and low-density lipoprotein cholesterol
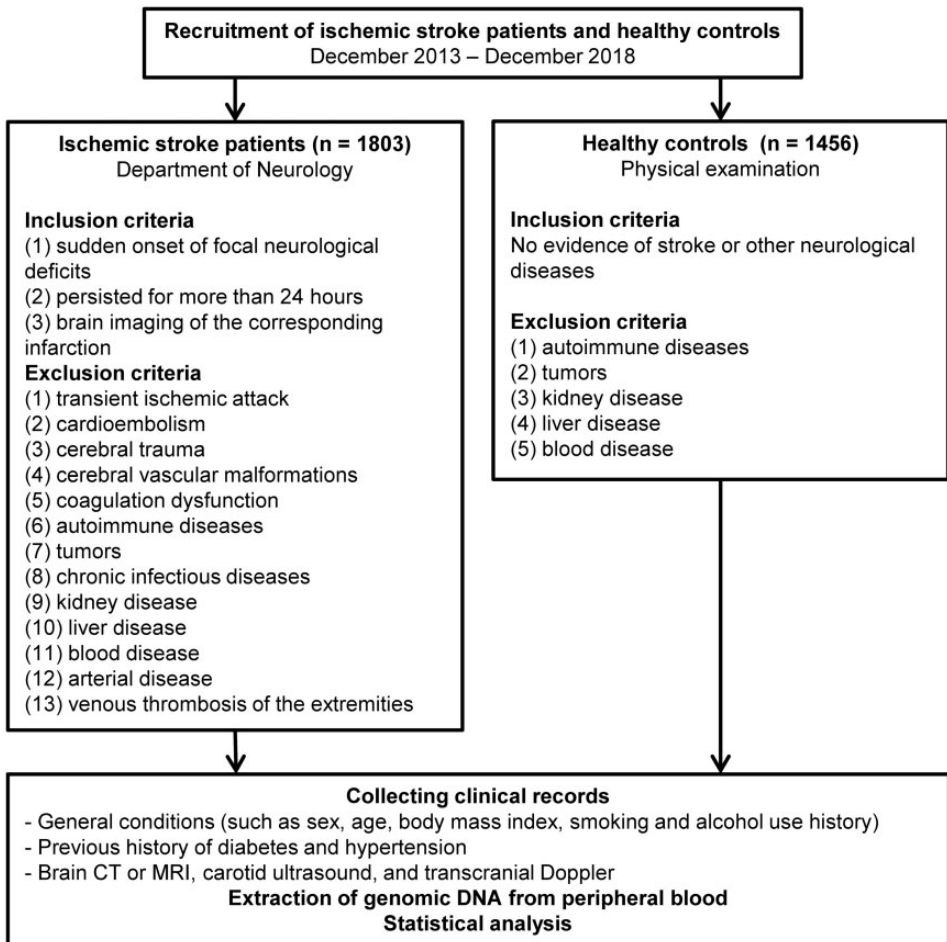

Figure 1 Flowchart of this study.
CT, computed tomography; MRI, magnetic resonance imaging.

Table 3. Characteristics and risk factors for stroke

Variable	Cases (%)	Controls (%)	p value
Age ($\leq$60/>60 years)	1083 (60.1)/720 (39.9)	921 (63.3)/535 (36.7)	0.065
Sex (male/female)	1032 (57.2)/771 (42.8)	826 (56.7)/630 (43.3)	0.776
BMI ($\leq$22.9/>22.9)	939 (52.1)/864 (47.9)	844 (58.0)/612 (42.0)	0.01
Diabetes mellitus	501 (27.8)/1302 (72.2)	268 (18.4)/1188 (81.6)	<0.001
Hypertension	1136 (63.0)/667 (37.0)	623 (42.8)/833 (57.2)	<0.001
Preexisting medication (aspirin)	194 (10.8)/1609 (89.2)	–	–
Preexisting medication (statins)	99 (5.5)/1704 (94.5)	–	–
Thrombolytic therapy	458 (25.4)/1345 (74.6)	–	–
Family history	134 (7.4)/1669 (92.6)	46 (3.2)/1410 (96.8)	<0.001
History of smoking	750 (41.6)/1053 (58.4)	425 (29.2)/1031 (70.8)	<0.001
History of alcohol use	362 (20.1)/1441 (79.9)	208 (14.3)/1248 (85.7)	<0.001
Hyperlipidemia	820 (45.5)/983 (54.5)	586 (40.2)/870 (59.8)	<0.001

BMI, body mass index.

levels, and lower high-density lipoprotein levels than controls. The patients were age- and sex-matched, implying that the sample was representative.

Genetic polymorphisms and effects on stroke risk

The genotype distributions of the two polymorphisms in IS cases and controls complied with the Hardy–Weinberg equilibrium. The frequency of rs10739971 genotype AA was significantly higher in patients (21.3%) compared with controls (17.0%) (OR = 1.330, CI = 1.081–1.637, p = 0.007) (Table 4). We further analyzed the frequencies in dominant (OR = 1.173, CI = 1.007–1.367, p = 0.041) and recessive models (OR = 1.246, CI = 1.037–1.497, p = 0.019). These results indicated that genotype AA might be associated with an increased risk of IS. Allele analysis also showed a significant difference in the frequency of the A allele (OR = 1.189, CI = 1.077–1.312, p < 0.001), suggesting that the A allele of rs10739971 was a possible risk factor for IS.

Regarding the rs17276588 pri-let-7f-2 polymorphism, the genotype frequencies of the GG, GA, and AA genotypes in the patients were 48.8%, 20.3%, 30.9%, respectively, compared with 81.0%, 8.9%, and 10.1% among the controls, with significant differences between patients and controls for AA (OR = 4.257, CI = 3.437–5.273, p < 0.001) and GA (OR = 4.181, CI = 3.260–5.362, p < 0.001) (Table 4). The results of the dominant and recessive models also indicated that genotypes AA and GA might be risk factors for IS. The frequency of the A allele differed significantly between the groups (OR = 4.106, CI = 3.632–4.641, p < 0.001), suggesting that the A allele of rs17276588 may be a risk factor for IS.

Given that rs17276588 is located on the X chromosome, we analyzed its allele and genotype frequencies in both sexes (Table 5). No GA genotype was found in men because men only have a single X chromosome. The A allele frequency differed significantly between patients and controls in men (OR = 5.032, CI = 4.296–5.894, p < 0.001), in accordance with the genotype analysis, showing an increased risk for AA compared with GG (OR = 4.403, CI = 3.475–5.578, p < 0.001). Similar to the overall population, the AA genotype differed between patients and controls in women (OR = 3.929, CI = 2.207–6.992, p < 0.001), but the OR in men was obviously higher than that for the whole

Table 4. Allele and genotype frequencies of genetic polymorphisms among cases and controls and their main effects on stroke risk.

SNP	Cases	%	Controls	%	OR (95% CI)*[a]	P value[b]
rs10739971 genotype						
GG (ref)	549	30.4	505	34.7	1.00 (ref)	
GA	870	48.3	703	48.3	1.116 (0.949–1.313)	0.185
AA	384	21.3	248	17.0	**1.330 (1.081–1.637)**	**0.007**
Dominant effect						
GG (ref)	549	30.4	505	34.7	1.00 (ref)	
GA + AA	1254	69.6	951	65.3	**1.173 (1.007–1.367)**	**0.041**
Recessive effect						
GA + GG (ref)	1419	78.7	1208	83.0	1.00 (ref)	
AA	384	21.3	248	17.0	**1.246 (1.037–1.497)**	**0.019**
rs10739971 allele						
G (ref)	1968	54.6	1713	58.8	1.00 (ref)	
A	1638	45.4	1199	41.2	**1.189 (1.077–1.312)**	**<0.001**
rs17276588 genotype						
GG (ref)	879	48.8	1180	81.0	1.00 (ref)	
GA	366	20.3	129	8.9	**4.181 (3.260–5.362)**	**<0.001**
AA	558	30.9	147	10.1	**4.257 (3.437–5.273)**	**<0.001**
Dominant effect						
GG(ref)	879	48.8	1180	81.0	1.00 (ref)	
GA + AA	924	51.2	276	19.0	**4.225 (3.579–4.987)**	**<0.001**
Recessive effect						
GA + GG (ref)	1245	69.1	1309	89.9	1.00 (ref)	
AA	558	30.9	147	10.1	**4.008 (4.233–4.969)**	**<0.001**
rs17276588 allele						
G (ref)	2464	68.3	2436	83.7	1.00 (ref)	
A	1142	31.7	476	16.3	**4.106 (3.632–4.641)**	**<0.001**

*ORs and 95% CIs calculated by logistic regression.
[a,b]Adjusted OR(95%CI) and p value adjusted for age, sex, body mass index, diabetes mellitus, hypertension, history of smoking, history of alcohol use, family history and hyperlipidemia.
SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

population, making the AA genotype a greater risk factor for IS in the male population. The outputs of the dominant (OR = 3.920, CI = 3.094–4.966, p < 0.001) and recessive (OR = 2.448, CI = 1.385–4.329, p = 0.002) models also suggested that AA genotype might be a risk factor and the A allele might be a risk allele (OR = 3.060, CI = 2.514–3.725, p < 0.001).

Gene–environment interactions

We analyzed the associations between genetic polymorphisms and risk-factor exposure. There was no multiplicative interaction between the rs17276588 polymorphism in either sex and a history of alcohol use (Table 6), but there was an additive effect of these two factors in the overall population, with a positive RERI and an almost qualified AP (RERI = 2.164, CI = 0.283–4.045; AP = 0.301, CI = −0.026 to 0.627). Individuals meeting both conditions were more susceptible to IS, indicating an overall synergistic effect between rs17276588 polymorphism and a history of alcohol use. The results stratified by alcohol exposure (Table 7) suggested that the significant

Table 5. Allele and genotype frequencies of genetic polymorphisms among cases and controls and their main effects on stroke risks in both sexes.

Sex	SNP	Cases		%	Controls	%	OR (95% CI)*[a]	p value[b]
Male	rs17276588 genotype							
	GG (ref)		532	51.6	696	84.3	1.00 (ref)	
	AA		500	48.4	130	15.7	**4.403 (3.475–5.578)**	**<0.001**
	rs17276588 allele							
	G (ref)		1064	51.6	1392	84.3	1.00 (ref)	
	A		1000	48.4	260	15.7	**5.032 (4.296–5.894)**	**<0.001**
Female	rs17276588 genotype							
	GG(ref)	347		45.0	484	76.8	1.00 (ref)	
	GA	366		47.5	129	20.5	**3.919 (3.061–5.016)**	**<0.001**
	AA	58		7.5	17	2.7	**3.929 (2.207–6.992)**	**<0.001**
	Dominant effect							
	GG (ref)	347		45.0	484	76.8	1.00 (ref)	
	GA+AA	424		55.0	146	23.2	**3.920 (3.094–4.966)**	**<0.001**
	Recessive effect							
	GA+GG (ref)	713		92.5	613	97.3	1.00 (ref)	
	AA	58		7.5	17	2.7	**2.448 (1.385–4.329)**	**0.002**
	rs17276588 allele							
	G (ref)	1060		68.7	1097	87.1	1.00 (ref)	
	A	482		31.3	163	12.9	**3.060 (2.514–3.725)**	**<0.001**

*ORs and 95% CIs calculated by logistic regression.
[a,b]Adjusted OR(95%CI) and p value, adjusted for age, body mass index, diabetes mellitus, hypertension, history of smoking, history of alcohol use, family history and hyperlipidemia.
SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Table 6. Multiplicative model for interaction between genetic polymorphisms and history of alcohol use on risk of ischemic stroke.

Genotype	Risk factor exposure	Male		Female	
		OR (95% CI)*	p value	OR (95% CI)*	p value
rs10739971	History of alcohol use	0.744 (0.434–1.275)	0.282	0.751 (0.420–1.340)	0.332
rs17276588		1.136 (0.594–2.171)	0.700	0.770 (0.409–1.448)	0.417

*ORs and 95% CIs calculated by logistic regression.
OR, odds ratio; CI, confidence interval.

association depended more on the polymorphism (wild-type or not) than on alcohol status in the female population (OR = 3.766, CI = 2.906–4.879, p < 0.001), but alcohol use increased the risk in women (OR = 5.345, CI = 3.144–9.085, p < 0.001). The GA and AA genotypes differed significantly between patients and controls in both men and women, irrespective of

alcohol use, supporting the possibility that the A allele was a risk factor for IS.

Gene–gene interactions

We determined the additive and multiplicative effects of the SNPs in both sexes using a dominant model (Table 8), and showed that the two SNPs had a synergistic interaction.

Table 7. Association between rs17276588 polymorphisms and risk of ischemic stroke stratified by alcohol exposure in both sexes.

rs17276588 genotype	Alcohol status	Male				Female			
		Cases (%)	Controls (%)	OR (95%CI)*a	p value b	Cases (%)	Controls (%)	OR (95%CI)*a	p value b
GG	No	422 (40.9)	611 (74.0)	1.00 (ref)		274 (35.5)	397 (63.0)	1.00 (ref)	
GA+AA	No	406 (39.3)	114 (13.8)	**6.156 (3.457–10.963)**	**<0.001**	339 (44.0)	126 (20.0)	**3.766 (2.906–4.879)**	**<0.001**
GG	Yes	110 (10.7)	85 (10.3)	**1.505 (1.064–2.129)**	**0.021**	73 (9.5)	87 (13.8)	1.119 (0.773–1.618)	0.551
GA+AA	Yes	94 (9.1)	16 (1.9)	**4.456 (3.454–5.749)**	**<0.001**	85 (11.0)	20 (3.2)	**5.345 (3.144–9.085)**	**<0.001**

*ORs and 95% CIs calculated by logistic regression.
a,bAdjusted OR(95%CI) and p value adjusted for age, body mass index, diabetes mellitus, hypertension, history of smoking, family history and hyperlipidemia.
OR, odds ratio; CI, confidence interval.

There were significant interactions in the multiplicative model in both men (OR = 1.624, CI = 1.022–2.580, p = 0.040) and women (OR = 2.155, CI = 1.269–3.663, p < 0.001), but the interaction was more significant and the OR was higher in women, suggesting a greater effect in women. There were also significant interactions between the polymorphisms in both men (OR = 2.705, CI = 1.979–3.696, p < 0.001) and women (OR = 4.610, CI = 3.322–6.397, p < 0.001) in the additive model. The results of the dominant model separated by sex may account for the sex differences in the multiplicative model.

The combined effects of the two polymorphisms in men and women are shown in Table 9. In men, mutated rs17276588 genotypes combined with either wild-type GG (OR = 2.962, CI = 2.009–4.366, p = 0.028) or mutated GA+AA (OR = 4.828, CI = 3.481–6.695, p < 0.001) in rs10739971 were all significant and the rs10739971 A allele had a higher OR, while any rs10739971 genotype combined with wild-type rs17276588 was not significant. In women, any single mutation in either SNP affected the risk of IS. From Table 10, stratified by rs10739971, the OR was >1 in both sexes, with a higher IS risk in females than in males; when stratified by rs17276588, mutated types in males (OR = 1.584, CI = 1.017–2.469, p = 0.042) and wild-type in females (OR = 1.705, CI = 1.232–2.359, p < 0.001) were significant in a dominant model of rs10739971, and the relatively small p value indicated a meaningful trend for GA+AA in females.

Discussion

The present study evaluated associations between two pri-miRNA SNPs (pri-let-7f-1 rs10739971 and pri-let-7f-2 rs17276588) and the risk of IS in a northern Chinese Han population. To the best of our knowledge, this is the first study to investigate these

Table 8. Multiplicative and additive models of association between two single nucleotide polymorphisms and risk of ischemic stroke.

SNP	Male		Female	
	OR (95%CI)*	p value	OR (95%CI)*	p value
rs10739971 (GA or AA vs GG)	1.561 (1.048–2.325)	0.028	0.810 (0.526–1.247)	0.338
rs17276588 (GA or AA vs GG)	5.978 (4.500–7.941)	<0.001	3.238 (2.463–4.256)	<0.001
Multiplicative model	**1.624 (1.022–2.580)**	**0.040**	**2.155 (1.269–3.663)**	**<0.001**
Additive model	**5.746 (4.207–7.849)**	**<0.001**	**5.652 (4.028–7.932)**	**<0.001**

*ORs and 95% CIs calculated by logistic regression.
SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

relationships in a large population (3259 subjects, 1803 patients and 1456 controls), as well as analyzing an X-linked locus, rs17276588, with the aim of exploring sex differences in IS risk. The findings indicated that both rs10739971 and rs17276588 polymorphisms were likely to be linked to an increased risk of IS. Individuals with the AA genotype of either SNP may have a higher risk of developing IS, with the A allele being a high-risk allele. The rs17276588 polymorphism tended to interact with a history of alcohol use, and alcohol use increased the risk of IS associated with rs17276588 mutation in women, indicating a synergistic effect. These results suggest that individuals with a high-risk genotype or allele, particularly women, should possibly avoid alcohol use. We also explored the interaction between the SNPs and demonstrated multiplicative and additive effects in both males and females. Certain groups of genotypes had combined effects, especially when several risk mutations occurred in the same individual. The lack of any GA genotype in males led to a slight difference in rs17276588, but the risk allele remained unchanged.

Let-7 family members have been widely studied as tumor suppressors. Let-7, as a ligand, has been reported to curb glioma growth via Toll-like receptor 7 in the brain, and was associated with microglial function.[28] The let-7 family has also been

investigated in relation to various neurological diseases and has been correlated with neurodegenerative disorders[29], while let-7b-5p levels in cerebrospinal fluid were reduced in patients with progressive multiple sclerosis.[30] Let-7b may be involved in the regulation of hepatic stellate cells[31], and let-7 family members have also been shown to suppress inflammation.[32–34] Our previous study on let-7a suggested a relationship between the rs1143770 and rs629367 SNPs on chromosome 11 and IS,[24] thus laying the foundations for the current study.

As a let-7 family member, let-7f plays a key role in many pathological processes during tumor development, including cell migration[35] and differentiation.[36] It was also shown to be involved in endothelial function and angiogenesis,[37] and a let-7f antagomir showed a neuroprotective effect.[38] Let-7f expression increased gradually during brain development and was shown to promote the differentiation of neural stem cells in rat models,[39] establishing a potential association between let-7f and brain disease. Another study showed that inhibition of let-7f increased the expression of the antiangiogenic protein, thrombospondin-1,[40] suggesting that increased expression of let-7f in the brain increased the risk of thrombosis. An antagomir to let-7f provided neuroprotection in an ischemic model by inhibiting let-7f expression and boosting the endogenous

Table 9. Combined effects of rs10739971 and rs17276588 polymorphisms on ischemic stroke risk.

		Male				Female			
rs10739971	rs17276588	Cases (%)	Controls (%)	OR (95%CI)*a	p value b	Cases (%)	Controls (%)	OR (95%CI)*a	p value b
GG	GG	189 (18.3)	241 (29.2)	1.00 (ref)		85 (11.0)	175 (27.8)	1.00 (ref)	
GA+AA	GG	343 (33.2)	455 (55.1)	0.889 (0.692–1.142)	0.357	262 (34.0)	309 (49.0)	**1.680 (1.228–2.297)**	**0.001**
GG	GA+AA	153 (14.8)	53 (6.4)	**2.962 (2.009–4.366)**	**<0.001**	122 (15.8)	36 (5.7)	**6.792 (4.286–10.763)**	**<0.001**
GA+AA	GA+AA	347 (33.6)	77 (9.3)	**4.828 (3.481–6.695)**	**<0.001**	302 (39.2)	110 (17.5)	**5.282 (3.739–7.461)**	**<0.001**

*ORs and 95% CIs calculated by logistic regression.
a,bAdjusted OR(95%CI) and p value adjusted for age, body mass index, diabetes mellitus, hypertension, history of smoking, history of alcohol use, family history and hyperlipidemia.
OR, odds ratio; CI, confidence interval.

neuroprotective agent, insulin-like growth factor-1, which plays an important role in the evolution of acute cerebral ischemia.[41] In addition, anti-let-7f oligonucleotides diminished cortical and striatal infarcts and preserved sensorimotor function and interhemispheric neural integration.[38] The endothelial function of let-7f was analyzed in young stroke patients, and it was considered to act as a biomarker in the diagnosis and prognosis of cerebral IS.[42]

The fact that pri-miRNAs affect miRNA expression via Drosha, the catalytic subunit of the microprocessor complex, suggests a possible underlying mechanism.[43] Previous studies of pri-let-7f rs10739971 and rs17276588 SNPs mostly focused on distinct types of cancer: Yuan et al. found that decreased transcription of rs17276588 A allele led to reduced levels of let-7f and a higher risk of colorectal cancer,[23] while pri-let-7f-1 rs10739971 polymorphism together with *ERCC6* and *PGC* polymorphisms served as a predictive model for the risk of gastric cancer.[44] Few studies have investigated the direct roles of these two SNPs in IS; however, the GA and AA variant genotypes of rs17276588 were associated with an enhanced risk of metabolic syndrome,[45] which is a multi-component disease caused by abnormal metabolic system abnormalities, leading to multiple clinical disorders including diabetes and dyslipidemia.[46] The influence of rs17276588 SNPs on metabolism not only sheds light on the prospective link between SNPs and IS, but also helps to explain the sex-related differences in its effects, via hormonal effects.

In this study, we assumed that rs10739971 and rs17276588 polymorphisms were associated with IS risk and tested this hypothesis in a hospital-based case–control study. We carried out statistical analysis using dominant and recessive models to estimate the mutations and their corresponding traits, and examined gene–environment and gene–gene interactions,

Table 10. Stratified odds ratios for rs10739971 and rs17276588 with respect to the risk of ischemic stroke.

Stratum	SNP	Male		Female	
		OR (95%CI)[*a]	p value[b]	OR (95%CI)[*a]	p value[b]
Stratified by **rs10739971**					
GA or AA	rs17276588 (AA vs GG)	**1.579 (1.056–2.361)**	**0.026**	**2.373 (1.819–3.096)**	**<0.001**
GG	rs17276588 (AA vs GG)	**2.656 (1.955–3.608)**	**<0.001**	**6.813 (4.271–10.869)**	**<0.001**
Stratified by **rs17276588**					
GA or AA	rs10739971 (GA or AA vs GG)	**1.584 (1.017–2.469)**	**0.042**	0.683 (0.448–1.042)	0.077
GG	rs10739971 (AA vs GG)	0.997 (0.786–1.264)	0.979	**1.705 (1.232–2.359)**	**<0.001**

*ORs and 95% CIs calculated by logistic regression.
a,bAdjusted OR(95%CI) and p value adjusted for age, body mass index, diabetes mellitus, hypertension, history of smoking, history of alcohol use, family history and hyperlipidemia.
SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

including both multiplicative and additive effects, to determine any extra impact on IS risk. However, it was not possible to reach a definite conclusion, despite the relatively large population involved in this study. This study had some limitations. First, we focused on a northern Chinese Han population, and more data are needed, especially from different regions and races. Second, this study failed to determine the specific gene–disease mechanism. Third, we did not carry out *in vivo* and *in vitro* experiments to test the results. However, despite these shortcomings, the study also had several strengths, and the comprehensive analyses help to uncover the underlying relationship.

In conclusion, the results of the current study suggest that the AA genotypes and A alleles of pri-let-7f-1 rs10739971 and pri-let-7f-2 rs17276588 are associated with an increased risk of IS. There is also a gene–environment interaction and a slight sex difference in the effect of rs17276588, suggesting that women with the A allele should play close attention to their alcohol consumption. These results support the use of these two SNPs as valuable biomarkers for caution, and individuals with high-risk alleles should undergo regular examinations and be careful regarding other conventional risk factors, to reduce the occurrence of IS. Further studies with larger sample sizes are needed to confirm these results and to investigate the potential mechanisms underlying the relationship between let-7f and IS.

Availability of data and materials

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Authors' contributions

LYQ carried out this study and drafted the manuscript. YYW prepared the figures and interpreted the data. SMD and FL conceived the project. ZYH, WXZ, and YZW revised and finalized the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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