Metabolic Brain Disease (2019) 34:1011–1021
https://doi.org/10.1007/s11011-019-00417-0

ORIGINAL ARTICLE

Association of long noncoding RNA H19 polymorphisms with the susceptibility and clinical features of ischemic stroke in southern Chinese Han population

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Received: 13 December 2018 / Accepted: 7 April 2019 / Published online: 30 April 2019

Abstract

Stroke is the leading cause of death in China. Previous studies have demonstrated that long noncoding RNAs play important roles in ischemic stroke (IS). This study aimed to investigate long noncoding RNA H19 (lncRNA H19) expression in IS cases and the association between lncRNA H19 variants and IS risk and IS-related risk factors. A total of 550 IS cases and 550 controls were recruited for this study. LncRNA H19 expression was detected using quantitative real-time polymerase chain reaction. Genotyping was conducted by the Sequenom MassARRAY technology. LncRNA H19 level in peripheral blood of IS cases was significantly upregulated compared with healthy controls ($P = 0.046$). No significant association was observed between lncRNA H19 rs217727 and rs4929984 polymorphisms with IS risk in all genetic models, and rs217727-rs4929984 haplotypes are not associated with IS susceptibility. Further meta-analysis also implied that the rs217727 and rs4929984 polymorphisms were not associated with IS in Chinese population. However, rs4929984 is significantly associated with the diastolic blood pressure level of IS patients (additive model: $P_{adj} = 0.007$; dominant model: $P_{adj} = 0.013$), whereas rs217727 is associated with international normalized ratio (additive model: $P_{adj} = 0.019$; recessive model: $P_{adj} = 0.004$), prothrombin time activity level (additive model: $P_{adj} = 0.026$; recessive model: $P_{adj} = 0.004$), and homocysteine level (recessive model: $P_{adj} = 0.048$) in patients with IS. Our findings suggest that lncRNA H19 level may affect the occurrence of IS, and lncRNA H19 variants may influence blood pressure, coagulation function, and homocysteine metabolism of patients with IS in the southern Chinese Han population.

Keywords Long noncoding RNA H19 · Ischemic stroke · lncRNA H19 expression · Polymorphism · Risk

Introduction

Ischemic stroke (IS) accounts for approximately 87% of stroke occurrences worldwide (Mozaffarian et al. 2016), and is the third leading cause of death in developed countries (Feigin et al. 2015) and the most common cause of death in China (Chen et al. 2017). Approximately 1.12 million Chinese aged ≥20 years died of a stroke, and the age-standardized mortality rates per 100,000 person-years in aged ≥20 years were 56.5 for IS from September 1, 2012 to August 31, 2013 (Chen et al. 2017). The American Heart Association predicts that by 2030, the direct cost of caring for stroke patients will reach $183.13 million (Ovbiagele et al. 2013), and because of high mortality, disability, and utilization of healthcare resources, stroke can bring a heavy economic cost to the world (Roy-O'Reilly and McCullough 2014). Nowadays, thrombolytic therapy is the most approved treatment for IS, but its effectiveness is limited on many strict conditions, such as in the first 3 h of thrombolysis after stroke

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11011-019-00417-0) contains supplementary material, which is available to authorized users.

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onset and other contraindications to treatment (Roy-O'Reilly and McCullough 2014). Thus, understanding the underlying mechanisms of IS and providing more evidence for diagnosis and therapy is urgent.

Long noncoding RNAs (lncRNAs) are a cluster of transcripts that lie in the non-protein coding regions of genes and have been the focus of considerable attention in recent years. Approximately 70%–90% of the human genome was transcribed to RNA, and lncRNAs account for more than 68% in human transcriptome (Iyer et al. 2015). Studies have provided evidence that the regulated mechanisms of lncRNAs include epigenetic, transcriptional, post-transcriptional, and translational regulations (Sun et al. 2017). In the transcriptional level, lncRNAs located in the nucleus could regulate and modify chromosomes and lead to the alteration of gene expression (Bao et al. 2018). Moreover, lncRNAs residing in the cytoplasm may act as a competing endogenous RNA for microRNAs and proteins to protect their target genes from degradation (Rashid et al. 2016). A growing number of studies have demonstrated that lncRNAs are involved in various multifactorial diseases in the human body, such as cancers, cardiovascular, autoimmune, and neurodegenerative diseases, and psychiatric disorders (Cipolla et al. 2018).

In the past years, lncRNAs have been involved in the development of IS. MALAT1, one of the first lncRNA identified to promote metastasis and proliferation of cancers, was increased significantly in oxygen-glucose deprivation (OGD) endothelial cells and middle cerebral artery occlusion mouse models of stroke and may act as a protective and healing properties of brain ischemia (Bao et al. 2018; Zhang et al. 2016). LncRNA FosDT promotes ischemic brain injury by binding chromatin-modifying proteins coREST and Sin3a to enable the formation of REST complex, providing a promising clue that lncRNA may be a potential therapy for post-stroke brain injury (Mehta et al. 2015). Moreover, lncRNA ANRIL was increased in rats with diabetes mellitus combining with cerebral infarction (Zhang et al. 2017). Increasing lncRNA ANRIL expression or mutation is also associated with stroke (Amouyel 2012). A large number of studies have shown that variants within chromosome 9p21.3, which overlaps with the lncRNA ANRIL (Kim et al. 2014) are associated with cardiovascular disease and IS risks (Chen et al. 2014; Zhang et al. 2012). For example, lncRNA ANRIL rs2383207 and rs1333049 are significantly associated with IS risk (Yang et al. 2018), and GG genotype of lncRNA ANRIL rs10757278 significantly increases stroke risk and recurrence in the Chinese population (Zhang et al. 2012), which suggests that lncRNAs may serve as a novel genetic marker for stroke.

Long noncoding RNA H19 (lncRNA H19) is a 2.3 kb RNA coded by *H19* gene, which plays a pivotal role in the embryonic development and growth control (Gabory et al. 2010). After birth, lncRNA H19 is rarely expressed in human tissues except in the cardiac and skeletal muscles (Gabory et al. 2010). An abnormal expression of lncRNA H19 in the postnatal period was reported to be involved in tumors, such as the bladder, gastric, colorectal, and breast cancers (Luo et al. 2013; Soudyab et al. 2016; Sun et al. 2016). In addition, studies have found an aberrant re-expression of lncRNA H19 in atherosclerotic plaque (Han et al. 1996) and rat vascular smooth muscle cells after a carotid artery injury (Kim et al. 1994). LncRNA H19 is also expressed abnormally in a status of hypoxia, which works as a stimulus for cerebral ischemia and reperfusion (I/R) injury (Matouk et al. 2010). Recently, the abnormal lncRNA H19 expression is significantly correlated with the risk factors of IS, such as blood pressure (BP) (Tragante et al. 2014) and coronary artery disease (CAD) (Wang et al. 2017a). Significantly upregulated levels of lncRNA H19 in stroke patients, animals, and cell models of IS were also detected (Wang et al. 2017a, b). LncRNA H19 polymorphisms are reported to participate in the regulation of lncRNA H19 expression (Yang et al. 2015), hence, more attention has been given to those polymorphisms. For example, the single-nucleotide polymorphism (SNP) rs217727 is correlated with the susceptibility of oral squamous cell carcinoma (Guo et al. 2017), and with the risk of CAD (Gao et al. 2015).

More recently, lncRNA H19 variants (rs217727, rs2067051, rs2251375, rs492994, rs2839698 and rs10732516) were reported in Chinese case control studies (Wang et al. 2017a; Zhu et al. 2018). Wang et al. (2017a) found the rs217727 and rs4929984 variants are associated with the risk of ischemic stroke (152 IS patients and 150 controls) and the minor alleles of rs217727 (T) and rs4929984 (A) increased the risk of IS. Even though Zhu et al. (2018) obtained a significant association of rs217727 with ischemic stroke, they found the alleles of rs217727(T/C) in patients with IS were not differ from controls. These findings are compelling but still divergent. To further ascertain the roles of lncRNA H19 and its variants in the development of ischemic stroke, in the present study, we examine the lncRNA H19 expression in the patients with IS, investigate the genetic association of lncRNA H19 SNPs rs217727 and rs4929984 with IS susceptibility and the relationship between lncRNA H19 variants and clinical parameters of patients with IS in Southern Chinese Han Population.

Material and methods

Subjects

A total of 550 unrelated IS patients and 550 unrelated healthy control subjects were obtained from the First Affiliated Hospital of Guangxi University of Chinese Medicine from September 2010 to June 2017. All subjects were of Han Chinese descent. According to the Fourth National

Conference on diagnostic criteria for cerebrovascular diseases in China, IS cases were diagnosed using computed tomography (CT) or magnetic resonance imaging (MRI) and were confirmed by at least two independent clinical neurologists. Patients with IS caused by other conditions, such as transient ischemic attacks, brain trauma, tumors, and cerebrovascular malformation, were excluded from our study. Control subjects were enrolled from volunteers in examination centers and inpatients with other slight diseases, and healthy controls have no history of IS and other cerebrovascular diseases. All participants provided informed consent, and this study was approved by the Ethics Committee of the Guangxi University of Chinese Medicine.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Peripheral blood leukocytes were collected in the morning, and total RNA were extracted using TRIzol™ Reagent (Invitrogen™, USA) according to the manufacturer's protocol. cDNA reverse transcription was conducted using PrimeScript RT Reagent Kit with gDNA Eraser (Takara, Japan) and T100™ Thermal Cycler (BIO-RAD, USA). Primers were designed and synthesized by Shanghai Sangon Biological Engineering (Shanghai, China).

LncRNA H19: 5'-TTCAAGCCACGACTCT-3′ (forward); 5'-GCTCACACTCACGCACACTC-3′ (reverse).

Primer sequences of reference gene GAPDH: 5'-AGTCCTTCCACGATACCAAAGT-3′ (forward); 5'-CATGAGAAGTATGACAACAGCCT-3′ (reverse).

qRT-PCR experiment was conducted on the Applied Biosystems 7500 Real-Time PCR Systems (Applied Biosystems™, USA) with an SYBR Premix Ex Taq™ II kit (TaKaRa) according to the manufacturer's instruction. qRT-PCR was performed at the condition of 95 °C for 30 s, 95 °C for 5 s, and then 60 °C for 34 s, with a total of 40 cycles. The relative expression of lncRNA H19 was calculated using $2^{-\triangle CT}$ method.

Genotyping

Two milliliters of fasting peripheral blood sample were collected from the studied individuals, and DNA was extracted using the DNA kit from Aidlab Biotechnologies (Beijing, China). Genotyping was conducted using the Sequenom MassARRAY technology by Bio Miao Biological Technology (Beijing, China). Primer sequences are shown as follows:

rs217727: 5'-ACGTTGGATGAAAGACACCATCGGAACAGC-3′ (forward), 5'ACGTTGGATGAGCTCTGGGATGATGTGGTG-3′ (reverse).

rs4929984: 5'-ACGTTGGATGTTTTGCAGAGGCGGTTTCAC-3′ (forward), 5'ACGTTGGATGATGCATGGGCTCCTAGACAG-3′ (reverse).

To confirm the quality of genotyping results, 5% of the DNA samples were selected to replicate detection and 100% consistency was obtained.

Clinical biochemical markers measurement

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a calibrated mercury sphygmomanometer in the morning. The Mindray-6900 automatic blood corpuscle analyzer was used to measure platelet (PLT) level; activated partial thromboplastin time (APTT), D-dimer (D-D), fibrinogen (FIB), international normalized ratio (INR), prothrombin time (PT), prothrombin time activity (PTA), and thrombin time (TT) were detected using a StAgO Capact blood coagulation analyzer. The levels of C-reactive protein, homocysteine (Hcy), and uric acid (UA) were measured using the Hitachi 7600 automatic biochemistry analyzer. All measurements were completed by the First Affiliated Hospital of Guangxi University of Chinese Medicine according to strict laboratory procedures.

Statistical analysis

Genetic association analysis was performed by PLINK software. χ^2 goodness-of-fit test was performed to determine Hardy–Weinberg Equilibrium (HWE). The distributions of genotype frequency between IS and control groups were compared using chi-square test. Non-conditional logistic regression analysis was used to evaluate the association of each loci with IS under different genetic models (additive, dominant, recessive, and allelic models). The generalized linear model

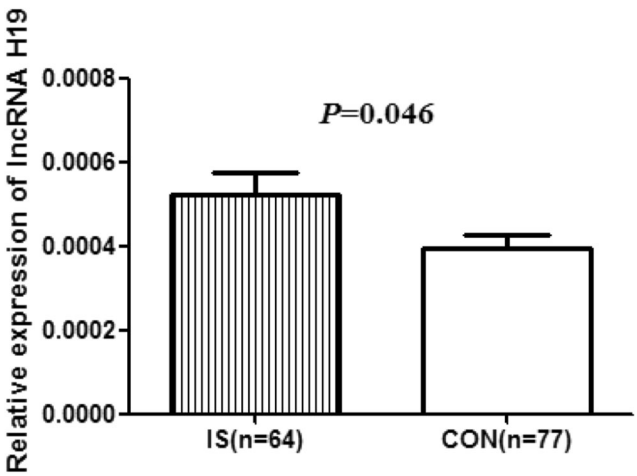

Fig. 1 Comparison of relative expression of lncRNA H19 in patients and healthy controls. IS: ischemic stroke, CON: healthy controls

Table 1 Hardy-Weinberg equilibrium test for the healthy controls

SNP_ID	Allele (minor/major)	IS			control			P_{HWE}	χ2	*P*
		A_1A_1	A_1A_2	A_2A_2	A_1A_1	A_1A_2	A_2A_2			
rs217727	A/G	38	221	291	47	204	298	0.1609	1.715	0.424
rs4929984	A/C	81	243	222	80	257	208	1.000	0.853	0.653

A1: minor allele-, A2: major allele-; P_{HWE}: HWE for control subjects

was used to determine the association between SNPs and clinical biochemical parameters. The relative expressions of lncRNA H19 in IS and control groups were compared through Student's t test, and the comparison of classified variables used chi-square test. These analyses were performed on the SPSS16.0 software. All significant results were set as two-tailed test and *P* value lower than 0.05.

Meta-analysis

A meta-analysis was conducted to study the association of lncRNA H19 variants with IS susceptibility. Heterogeneity test was determined by the Q-test and I^2 statistics, and if the $I^2 > 50\%$ and $P > 0.10$, data were merged using the fixed-effect model (Mantel–Haenszel method). Otherwise, the random-effect model (The DeSimonian and Laird method) was used. The odds ratio (OR) with 95% confidence interval (CI) represents the effect strength. The above analysis was conducted using Stata 12.0. All tests were two tailed and a statistical significance was set as $P < 0.05$.

Results

Baseline characteristics of the study subjects

A total of 550 IS patients (306 males and 244 females) and 550 healthy controls (293 males and 257 females) were included in our study. The mean age was 70.10 ± 8.82 in patients with IS and 69.23 ± 9.68 in control subjects. Age ($t = -1.560$, $P = 0.119$) and gender ($\chi^2 = 0.619$, $P = 0.431$) in IS cases and controls did not differ significantly.

LncRNA H19 expression in study subjects

We selected 64 IS patients and 77 healthy controls to determine lncRNA H19 expression. Figure 1 shows that compared with the control group, a significant upregulation of lncRNA H19 expression in peripheral blood in IS patients was obtained ($t = 2.023$, $P = 0.046$).

Association analysis of lncRNA H19 variants with IS risk

A total of 550 IS patients and 550 healthy controls were genotyped, but 4 in IS group of rs4929984, 1 in control group of rs217727 and 5 in controls of rs4929984 were failed to genotype. Thus, 550 IS patients and 549 controls for rs217727 and 546 IS patients and 545 controls for rs4929984 were finally analyzed in the genetic association of lncRNA H19 polymorphisms with IS. The distributions of genotypes of rs217727 and rs4929984 in healthy controls were in Hardy–Weinberg Equilibrium ($P > 0.05$). Table 1 shows that no significant difference was found in the distributions of genotype frequency of lncRNA H19 variants between IS and healthy controls (rs217727: $\chi^2 = 1.715$, $P = 0.424$; rs4929984: $\chi^2 = 0.853$, $P = 0.653$). What's more, rs217727 and rs4929984 showed no significant association with IS susceptibility under the additive, dominant, recessive, and allelic models (Table 2).

Haplotype association analysis

The rs4929984 (A/C) and rs217727 (A/G) constituted C-A, A-G, and C-G haplotypes. No significant association between three haplotypes and IS susceptibility was observed ($P > 0.05$, Table 3).

Meta-analysis

Four studies (including our study) were included in this meta-analysis. A total of 1269 IS cases and 1251 controls for rs217727, and 698 IS cases 695 controls for rs4929984 were analyzed (Supplemental Table 1). The genotypic distributions of rs217727 and rs4929984 in total controls were in accordance with HWE ($P > 0.05$). After pooling all data, both the SNPs rs217727 and rs4929984 were not significantly associated with the risk of IS in the four genetic models (all $P > 0.05$, Table 4).

Association analysis of lncRNA H19 variants and risk factors of patients with IS

As presented in Table 5, rs4929984 was significantly associated with the DBP level of IS patients in the additive (β: −2.02[−3.59to–0.45], $P = 0.012$) and dominant models (β: −2.62[−4.86to–0.38], $P = 0.022$). The significant association

Table 2 Association of lncRNA H19 variants rs217727 and rs4929984 with IS risk

SNP_ID	Additive model				Dominant model				Recessive model				Allelic model	
	OR (95%CI)	*P*	Adjusted OR (95%CI)	P_{adj}	OR (95%CI)	*P*	Adjusted OR (95%CI)	P_{adj}	OR (95%CI)	*P*	Adjusted OR (95%CI)	P_{adj}	OR (95%CI)	*P*
rs217727 (A/G)	0.99 (0.82–1.20)	0.942	0.99 (0.82–1.20)	0.925	1.06 (0.83–1.34)	0.649	1.06 (0.83–1.34)	0.657	0.79 (0.51–1.24)	0.306	0.79 (0.50–1.23)	0.294	0.99 (0.82–1.20)	0.941
rs4929984 (A/C)	0.95 (0.80–1.13)	0.578	0.96 (0.81–1.14)	0.578	0.90 (0.71–1.15)	0.399	0.91 (0.71–1.16)	0.460	1.01 (0.72–1.42)	0.942	1.01 (0.73–1.42)	0.934	0.95 (0.80–1.13)	0.573

was still observed after adjustment for age and gender under the additive ($P_{adj} = 0.007$) and dominant models ($P_{adj} = 0.013$). As summarized in Table 6, a significant association between rs217727 and INR level of IS patients was obtained (additive β: 1.39[0.25–2.53], $P = 0.017$, $P_{adj} = 0.019$; recessive β: 4.18[1.38–6.97], $P = 0.004$, $P_{adj} = 0.004$), and rs217727 was also significantly associated with PTA parameter (additive β: −3.09[−5.63to–0.54], $P = 0.018$, $P_{adj} = 0.026$; recessive β: −9.51[−15.68to–3.32], $P = 0.003$, $P_{adj} = 0.004$). Moreover, rs217727 was significantly associated with Hcy level in patients with IS under the recessive model (β: 6.18[0.07–12.18], $P = 0.048$), and this association remained after the adjustment of age and gender ($P_{adj} = 0.048$, Table 7).

Discussion

Our findings showed that lncRNA H19 level was upregulated in IS patients compared with healthy controls. The lncRNA H19 rs217727 and rs4929984 polymorphisms are not significantly associated with IS susceptibility in the southern Chinese Han population. Further, rs4929984 is significantly associated with the DBP level of IS cases, and rs217727 is significantly correlated with INR, PTA, and Hcy levels in IS patients (Table 7).

Upon the investigation on the effects of lncRNA H19 level on IS, we found an increased lncRNA H19 level in patients with IS. This result was in agreement with the findings of Wang (Wang et al. 2017a, b), whose results indicated an upregulated lncRNA H19 level in stroke patients ($N = 36$), brain tissues of mice with cerebral I/R injury, and human neuroblastoma cell (SH-SY5Y cell) after induction by OGD/R. Generally, lncRNA H19 inhibits autophagy by regulating DUSP5-ERK1/2 axis under the cellular OGD/R model (Wang et al. 2017a). Excessive autophagy in cerebral ischemia may induce apoptosis and mediate neuronal death (Puyal and Clarke 2009), and the inhibition of lncRNA H19 decreases cerebral infarct volume and brain edema and neurological deficits (Wang et al. 2017b). These data indicated that lncRNA H19 has a pivotal effect on the occurrence of IS.

As an imprinted gene, lncRNA H19 is expressed mainly in the period of the embryo and it executes the function of controlling embryo growth by targeting another imprinted gene, that is, the insulin-like growth factor 2 (Wilkin et al. 2000). LncRNA H19 is located in chromosome 11p15.5, and rs217727 and rs4929984 lying in *H19* are associated with the birth weight of newborns (Adkins et al. 2010; Hewage et al. 2015). Previous Chinese studies have reported that lncRNA H19 variants rs217727 and rs4929984 are significantly correlated with IS risk in the northern Chinese population (Wang et al. 2017a; Zhu et al. 2018). Gao et al. (2015) found that lncRNA H19 rs217727 is the risk loci of CAD. However, we failed to find any significant association

Table 3 Haplotype association analysis of lncRNA H19 variants rs217727 and rs4929984 with IS

Haplotype		Frequency-case	Frequency-control	OR	*P*	Adjusted OR*	P_{adj}
C	A	0.27	0.27	1.00	0.980	1.00	0.960
A	G	0.37	0.38	0.95	0.549	0.96	0.602
C	G	0.36	0.35	0.35	0.555	1.05	0.595

P_{adj}: adjusted by sex, age

between lncRNA H19 variants and IS susceptibility. On one hand, after combining previous studies, the results of meta-analysis show that the SNPs rs217727 and rs4929984 are not associated with IS risk, indicating that the small sample size is the major reason for our inconsistency. On the other hand, we speculate that a possible reason may be the genetic heterogeneity in different regions in China. Evidence proved that the Chinese population from north to south carries different allele frequencies of some human diseases (Yuan et al. 2013). In the study of previous studies (Wang et al. 2017a; Zhu et al. 2018), their participants were enrolled from the northern part of China, and may have reached to inconsistent findings as compared with ours. Thus, studies with larger sample size and multi-region are warranted to explain the correlation of lncRNA H19 variants with IS.

To investigate the association between lncRNA H19 variants and clinical biochemical parameters of IS patients, we collected clinical-related markers of patients with IS and performed an association analysis. As a result, we observed a significant correlation between rs4929984 and DBP level of patients with IS. SBP and DBP were demonstrated to be significantly associated with IS in the past years (Liu et al. 2013; Verdecchia et al. 2015). To the best of our knowledge, hypertension is defined as SBP ≥ 140 mmHg and DBP ≥ 90 mmHg (Kearney et al. 2005) and hypertension has been identified as a risk factor of IS. In recent years, genome-wide association studies (GWAS) have identified a number of genes and risk variants like *SH2B3* rs3184504 associated with BP through a combination study of a large sample in Europe ($N = 133,661$), and these variants were confirmed to be strongly correlated with SBP and DBP in individuals of East Asian ($N = 29,719$), South Asian ($N = 23,977$), and African ($N = 19,775$) ancestry (International Consortium for Blood Pressure Genome-Wide Association et al. 2011). And genetic risk score assessing the association of the variants in aggregate BP was positively correlated with the incidence of stroke and CAD (International Consortium for Blood Pressure Genome-Wide Association et al. 2011). In addition, compared with the CC genotype, Feng et al. (2017) reported that *AMPD1* gene C34T polymorphism CT + TT genotype is significantly associated with decreased SBP of patients with cardiovascular diseases, which made one of the foundations for IS. rs4929984 is significantly associated with the blood pressure risk factor of IS, which suggests that lncRNA H19 variants involved in the development of IS may be related to the regulation of blood pressure.

Generally, abnormal blood coagulation plays a pivotal role in the pathogenesis of thrombus (Vazquez-Garza et al. 2017), which in turn leads to IS. Up to date, studies from GWAS and large sample subjects have identified plenty of risk loci and variants of thrombosis and IS (Chauhan and Debette 2016; Hinds et al. 2016). In investigating the correlation between gene variants and coagulation markers in stroke, Williams et al. (2013) found that *ABO* gene SNP rs505922 influencing blood coagulation is significantly associated with IS, with the T allele being a protective factor and the significance was confirmed in the subsequent subtype analysis of stroke. As a risk factor for atherosclerosis, ANRIL SNPs (rs10116277, rs7865618, rs564398, rs496892, and rs7044859) are significantly associated with IS and CAD (Cunnington et al. 2010)

Table 4 Association between rs217727 and rs4929984 polymorphisms and blood pressure levels in patients with IS

SNP_ID	Number of cases/controls	Genetic model	OR(95%CI)	*P*	I^2(%)	$P_{Heterogeneity}$
rs217727 (A/G)	1269/1251	Additive model	1.01(0.87–1.19)	0.829	27.8	0.251
		Dominant model	1.40(0.87–2.26)	0.162	85.4	0.001
		Recessive model	1.41(0.80–2.48)	0.239	81.5	0.004
		Allelic model	1.32(0.91–1.93)	0.147	88.9	0.000
rs4929984 (A/C)	698/695	Additive model	1.04(0.89–1.21)	0.631	0.0	0.447
		Dominant model	1.24(0.83–1.87)	0.294	83.0	0.003
		Recessive model	1.30(0.93–1.80)	0.123	58.9	0.088
		Allelic model	1.23(0.90–1.69)	0.194	86.1	0.001

Table 5 Association between rs217727 and rs4929984 polymorphisms and blood coagulation markers in patients with IS

SNP_ID	Model	SBP				DBP			
		β^a (95%CI)	Crude *P*	β^b(95%CI)	P_{adj}	β^a (95%CI)	Crude *P*	β^b(95%CI)	P_{adj}
rs217727 (A/G)	Additive	0.61 (−2.43–3.64)	0.695	0.63 (−2.41–3.66)	0.685	1.51 (−0.26–3.28)	0.094	1.52 (−0.19–3.24)	0.082
	Dominant	1.04 (−2.74–4.81)	0.590	1.03 (−2.74–4.82)	0.591	1.85 (−0.35–4.05)	0.100	1.74 (−0.4–3.88)	0.111
	Recessive	−0.39 (−7.82–7.05)	0.919	−0.23 (−7.68–7.21)	0.951	1.92 (−2.42–6.26)	0.386	2.45 (−1.77–6.66)	0.255
rs4929984 (A/C)	Additive	−1.09 (−3.80–1.62)	0.429	−1.17 (−3.89–1.55)	0.400	−2.02 (−3.59to-0.45)	0.012	−2.02 (−3.66to-0.60)	0.007
	Dominant	−1.19 (−5.04–2.67)	0.547	−1.31 (−5.19–2.57)	0.507	−2.62 (−4.86to-0.38)	0.022	−2.79 (−4.98to-0.60)	0.013
	Recessive	−1.97 (−7.29–3.36)	0.470	−2.01 (−7.34–3.33)	0.461	−2.80 (−5.90–0.31)	0.078	−2.91 (−5.93–0.10)	0.059

β^a: regression coefficient without adjusted; β^b: regression coefficient adjusted by age, sex; P_{adj}: adjusted by age, sex

although the underlying mechanism is unclear. LncRNA H19 has been reported aberrantly expressed in atherosclerosis plaque (Pan 2017), and we found a significant association between lncRNA H19 rs217727 and coagulation markers INR and PTA level, which suggests that coagulation dysfunction could be a reason for the involvement of lncRNA H19 polymorphism in the formation of thrombosis in IS occurrence. Functional studies need to verify our speculation.

In addition to BP and coagulation function, another risk factor, Hcy, also contributes to IS especially in hypertensive patients (Wang et al. 2014). Plasma Hcy level is commonly influenced by gene and variants. Zhao et al. (2017) investigated the association between the *MTHFR* C677T polymorphism and Hcy level in the incidence of IS and found that Hcy increases the risk of first stroke in subjects with the TT genotype, but this effect is significantly modified by *MTHFR* gene, indicating a significant gene–Hcy interaction in the occurrence of IS. Moreover, elevated Hcy-associated SNPs rs838133 and rs7422339 are correlated with IS (Cotlarciuc et al. 2014). A high Hcy level would tend the endothelial and vascular smooth muscle cells to injury, resulting in endothelial proliferation, activation of coagulation factors, and expression of plasminogen activator inhibitor, in turn, lead to PLT aggregation and subsequently IS (Hainsworth et al. 2016). Given that lncRNA H19 is involved in vascular endothelial cell function by regulating endothelial cell proliferation (Simion et al. 2018), lncRNA H19 rs217727 was significantly associated with the Hcy level of IS in this study, indicating that Hcy may be an intermediary between lncRNA H19 variations and the occurrence of IS. Taken together, lncRNA H19 might be involved in IS through the modification of blood pressure, coagulation function, and Hcy level. These findings need to be further verified.

Limitations

Several limitations need to be addressed. (i) This work is a single-center and retrospective study, and we only recruited Chinese Han population. Thus, selection bias may exist. Multi-center and prospective studies need to be conducted to demonstrate the association of lncRNA H19 with the occurrence of IS. (ii) Given that diverse IS subtypes may lead to different clinical endings, subtype analysis needs to be performed further. (iii) Although this study found an upregulation of lncRNA H19 level in 64 IS patients compared with 77 healthy controls and lncRNA H19 polymorphisms were associated with IS-related clinical parameters, our sample size was too small relative to the study of GWAS, and this size may not be sufficient to demonstrate the role of lncRNA H19 in IS. Hence, studies with large sample individuals are needed to verify our findings.

Table 6 Association of rs217727 and rs4929984 polymorphisms with blood CRP, Hcy and UA levels in patients with IS

Variables	SNP_ID	Additive				Dominant				Recessive			
		β^a (95% CI)	Crude P	β^b (95% CI)	P_{adj}	β^a (95% CI)	Crude P	β^b (95% CI)	P_{adj}	β^a (95% CI)	Crude P	β^b (95% CI)	P_{adj}
APTT	rs217727	0.69 (−0.28–1.66)	0.164	0.67 (−0.30–1.64)	0.178	0.61 (−0.60–1.83)	0.324	0.58 (−0.63–1.79)	0.349	1.79 (−0.60–4.18)	0.142	1.79 (−0.60–4.18)	0.142
	rs4929984	−0.20 (−1.07–0.68)	0.659	−0.14 (−1.01–0.73)	0.751	−0.62 (−1.86–0.62)	0.328	−0.50 (−1.74–0.75)	0.435	0.42 (−1.29–2.12)	0.633	0.39 (−1.31–2.09)	0.655
D-D	rs217727	−0.28 (−1.09–0.53)	0.501	−0.30 (−1.11–0.51)	0.468	−0.54 (−1.56–0.48)	0.300	−0.56 (−1.58–0.46)	0.282	0.36 (−1.60–2.32)	0.717	0.31 (−1.65–2.27)	0.758
	rs4929984	−0.27 (−1.01–0.47)	0.475	−0.24 (−0.98–0.50)	0.531	−0.46 (−1.51–0.58)	0.385	−0.40 (−1.46–0.65)	0.452	−0.15 (−1.60–1.30)	0.840	−0.14 (−1.59–1.31)	0.849
FIB	rs217727	0.05 (−0.10–0.20)	0.490	0.08 (−0.10–0.19)	0.534	0.04 (−0.14–0.23)	0.649	0.04 (−0.15–0.22)	0.675	0.15 (−0.21–0.51)	0.424	0.13 (−0.23–0.49)	0.484
	rs4929984	0.09 (−0.04–0.22)	0.184	0.10 (−0.04–0.23)	0.159	0.16 (−0.03–0.35)	0.104	0.17 (−0.02–0.36)	0.085	0.05 (−0.21–0.31)	0.712	0.05 (−0.21–0.31)	0.691
INR	rs217727	1.39 (0.25–2.53)	0.017	1.36 (0.22–2.51)	0.019	1.09 (−0.34–2.51)	0.137	1.06 (−0.37–2.48)	0.148	4.18 (1.38–6.97)	0.004	4.15 (1.35–6.94)	0.004
	rs4929984	−0.37 (−1.40–0.66)	0.482	−0.32 (−1.35–0.71)	0.544	−0.43 (−1.90–1.03)	0.561	−0.32 (−1.79–1.15)	0.671	−0.60 (−2.62–1.42)	0.562	−0.62 (−2.65–1.40)	0.545
PLT	rs217727	5.70 (−4.46–15.86)	0.272	6.07 (−6.06–16.10)	0.237	3.21 (−9.55–15.97)	0.622	3.65 (−8.95–16.26)	0.570	21.41 (−3.14–45.96)	0.088	21.96 (−2.30–46.22)	0.077
	rs4929984	5.94 (−3.20–15.08)	0.203	5.05 (−3.99–14.10)	0.274	4.50 (−8.50–17.50)	0.498	2.24 (−10.66–15.14)	0.734	14.39 (−3.59–32.37)	0.117	15.28 (−2.49–33.05)	0.092
PT	rs217727	0.24 (−0.66–1.14)	0.607	0.22 (−0.68–1.13)	0.627	0.43 (−0.70–1.56)	0.457	0.41 (−0.72–1.54)	0.476	−0.22 (−242–1.98)	0.845	−0.23 (−2.43–1.97)	0.838
	rs4929984	0.13 (−0.69–0.94)	0.761	0.16 (−0.66–0.97)	0.708	0.38 (−0.78–1.54)	0.520	0.45 (−0.71–1.61)	0.450	−0.23 (−1.82–1.37)	0.773	−0.24 (−1.84–1.35)	0.764
PTA	rs217727	−3.09 (−5.63to-0.54)	0.018	−2.85 (−5.35to-0.35)	0.026	−2.32 (−5.52–0.88)	0.155	−2.08 (−5.22–1.05)	0.194	−9.51 (−15.68to-3.32)	0.003	−9.02 (−15.08to-2.96)	0.004
	rs4929984	1.54 (−0.76–3.84)	0.189	1.17 (−1.08–3.44)	0.308	3.22 (−0.04–6.49)	0.053	2.43 (−0.79–5.66)	0.139	−0.19 (−4.71–4.33)	0.935	−0.07 (−4.50–4.35)	0.975
TT	rs217727	−0.42 (−1.84–1.01)	0.567	−0.44 (−1.88–0.98)	0.539	−0.84 (−2.63–0.95)	0.355	−0.88 (−2.67–0.91)	0.334	0.71 (−2.77–4.21)	0.687	0.68 (−2.81–4.17)	0.703
	rs4929984	−0.98 (−2.27–0.30)	0.135	−0.93 (−2.21–0.37)	0.161	−1.56 (−3.39–0.27)	0.096	−1.43 (−3.28–0.41)	0.128	−0.82 (−3.34–1.70)	0.524	−0.84 (−3.36–1.68)	0.512

β^a: regression coefficient without adjusted; β^b: regression coefficient adjusted by age, sex; P_{adj}: adjusted by age, sex

Table 7 Meta-analysis of rs217727 and rs4929984 polymorphisms and the susceptibility to ischemic stroke

Variables	SNP_ID	Additive				Dominant				Recessive			
		β^a (95% CI)	Crude *P*	β^b (95% CI)	P_{adj}	β^a (95% CI)	Crude *P*	β^b (95% CI)	P_{adj}	β^a (95% CI)	Crude *P*	β^b (95% CI)	P_{adj}
CRP	rs217727	−1.73 (−8.91–5.46)	0.639	−1.78 (−8.96–5.41)	0.629	−1.34 (−10.44–7.76)	0.774	−1.40 (−10.50–7.69)	0.763	−4.89 (−21.73–11.94)	0.570	−4. 95 (−21.79–11.89)	0.566
	rs4929984	−1.65 (−8.31–5.02)	0.629	−1.34 (−8.06–5.39)	0.698	1.06 (−8.36–10.47)	0.826	1.82 (−7.64–11.29)	0.706	−8.29 (−21.22–4.65)	0.212	−8.64 (−21.72–4.45)	0.198
Hcy	rs217727	1.49 (−1.06–4.03)	0.253	1.49 (−1.05–4.04)	0.251	0.66 (−2.57–3.90)	0.688	0.68 (−2.56–3.92)	0.683	6.13 (0.07–12.18)	0.048	6.13 (0.07–12.20)	0.048
	rs4929984	−1.00 (−3.36–1.35)	0.403	−0.94 (−3.29–1.42)	0.437	−0.96 (−4.26–2.35)	0.571	−0.77 (−4.09–2.55)	0.650	−2.07 (−6.76–2.62)	0.388	−2.18 (−6.87–2.52)	0.364
UA	rs217727	−6.91 (−19.93–7.49)	0.374	−6.92 (−20.37–7.54)	0.314	−4.36 (−21.57–12.85)	0.620	−5.65 (−22.54–11.25)	0.513	−20.07 (−53.11–12.97)	0.234	−19.38 (−510.84–13.07)	0.242
	rs4929984	−4.51 (−16.96–7.94)	0.478	−3.10 (−15.34–9.15)	0.620	−7.76 (−25.33–9.81)	0.387	−4.65 (−21.97–12.67)	0.599	−2.41 (−27.13–22.31)	0.849	−3.04 (−27.3–21.22)	0.806

β^a: regression coefficient without adjusted; β^b: regression coefficient adjusted by age, sex; P_{adj}: adjusted by age, sex

Conclusions

In summary, our findings suggested that the lncRNA H19 may have an effect on the occurrence of IS. LncRNA H19 rs217727 and rs4929984 polymorphisms, which in turn, may affect the level of blood pressure, coagulation function, and homocysteine metabolism of IS patients in the southern Chinese Han population. Functional and mechanism studies with a larger sample size are necessary to elucidate the underlying pathogenesis of lncRNA H19 in stroke.

Acknowledgements The present study was funded by the National Natural Science Foundation of China (Grant No.81573756), the Guangxi department of science and technology project (Grant No.1598012-12) and the Guangxi National Natural Science Foundation (Grant No.2017GXNSFDA198013).

Compliance with ethical standards

Conflict of interest There are no financial or other conflicts of interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of the Guangxi University of Chinese Medicine and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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