Research Square

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A genetic variant in the promoter of lncRNA MALAT1 is related to susceptibility of ischemic stroke

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Research

Keywords: Ischemic stroke, Polymorphism, Metastasis associated lung adenocarcinoma transcript 1

Posted Date: January 13th, 2020

DOI: https://doi.org/10.21203/rs.2.20757/v1

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Version of Record: A version of this preprint was published at Lipids in Health and Disease on April 1st, 2020. See the published version at https://doi.org/10.1186/s12944-020-01236-4.

Abstract

Background: Metastasis-associated lung adenocarcinoma transcript-1 (MALAT1) was aberrantly expressed in diverse diseases, and especially plays an important role in nerve injury including promotion of angiogenesis, inhibition of apoptosis and inflammation, regulation of autophagy, which are closely linked to the pathological processes of ischemic stroke (IS). However, the effect of genetic variation (single nucleotide polymorphisms, SNPs) of MALAT1 on IS have rarely been explored. This study aimed to investigate whether polymorphisms in promoter of MALAT1 were associated with the susceptibility to IS.

Methods: A total of 316 IS patients and 320 age-, gender-, and ethnicity-matched controls were enrolled in this study. Four polymorphisms in the promoter of MALAT1 (i.e., rsrs600231, rs1194338, rs4102217, and rs591291) were genotyped using a custom-by-design 48-Plex SNPscan kit.

Results: The rs1194338 C>A variant in MALAT1 promoter was associated with IS risk (AC vs. CC: adjusted OR = 0.623, 95% CI, 0.417-0.932, P = 0.021; AA vs. CC: adjusted OR = 0.474, 95% CI, 0.226-0.991, P = 0.047; Dominant model: adjusted OR = 0.596, 95% CI, 0.406-0.874, P = 0.008; A vs. C adjusted OR = 0.658, 95% CI, 0.487-0.890, P = 0.007). The Haplotype analysis showed that rs600231-rs1194338-rs4102217-rs591291 (A-C-G-C) had a 1.3-fold increase of IS risk (95% CI, 1.029-1.644, P=0.027). Logistic regression analysis identified some independent impact factors for IS including rs1194338 AC/AA, TC, TG, HDL-C, LDL-C, Apo-A1, Apo-B and NEFA($P < 0.05$).

Conclusions: These results suggest that the rs1194338 AC/AA genotypes may be a protective factor for IS.

Background

Stroke is a nervous system disease caused by cerebral blood circulation disorder and brain tissue damage with high fatality, disability and recurrence rate worldwide. Stroke has become the first cause of death In China, along with approximately 2.5 million new cases and 1.5 million deaths each year[1, 2]. The ischemic stroke (IS) accounts for about 87% of total cases [3]. Therefore, it is urgently required to explore etiology for meaningful targets. As we all known, the IS was a multifactorial complex disease. Traditional factors such as age, gender, obesity, hypertension, diabetes and smoking can only explain part of the IS risk [4–7]. Twins, familial aggregation and genome-wide association studies revealed that heredity was also a powerful factor in progression of IS[8–12].

Long non-coding RNAs (lncRNAs), with a length of more than 200 nucleotides, are emerging as key regulators of physiological and pathological processes[13]. Using RNA sequencing technology, a lot of lncRNAs were abnormally expressed after 16 h under oxygen-glucose deprivation (OGD) condition [14]. MALAT1, one of the most highly upregulated lncRNAs, was further confirmed to promote angiogenesis and autophagy while reduce apoptosis and inflammation both in vitro and in vivo[15–17]. For example, MALAT1 reduced ischemic cerebral damages by regulating 15-LOX1, VEGF and STAT3 related to angiogenesis[15], and acting a competing endogenous RNA for miR-26b to upregulate autophagy factor ULK2 expression directly[16]. Silencing of MALAT1 obviously increased expression of the proapoptotic and proinflammatory cytokines including Bim, IL-6, MCP − 1and E-selectin [17]. Taken together, MALAT1 may plays a protective role in IS, but the exact mechanism is still not fully known.

Currently, single nucleotide polymorphism (SNP) of lncRNAs have been proved to be associated with IS susceptibility, such as the rs217727 C > T and rs4929984 C > A in lncRNA H19, the rs2240183 C allele in promoter of lncRNA TUG1 [18–20]. It was reported that genetic variants in the promoter region can affect the expression, subcellular localization, structure stability, and ultimately involve in function and disease progression[21]. At the same time, studies also showed that polymorphisms in MALAT1 affect susceptibility and progression of diseases including hepatocellular cancer, lung adenocarcinoma and pulmonary arterial hypertension [22–24], but the impacts on IS have rarely been explored. To date, no study was conducted for the SNPs (rs600231, rs1194338, rs591291 and rs4102217) in promoter of MALAT1 with IS risk. Given the above, we

performed SNPs of MALAT1 gene from 636 samples (320 controls and 316 IS patients) in Chinese southwestern population, attempting to identify new candidates for the etiology of IS.

Methods

Study population

A total of 316 patients with IS and 320 controls were consecutively recruited from the Affiliated Hospital of Youjiang Medical University for Nationalities, Guangxi, China, between March 2015 and July 2018. All subjects were native people living in Guangxi province who were unrelated with Han Chinese. The IS patients were diagnosed based on clinical manifestations, physical examination and cranial computed tomography or magnetic resonance imaging. Exclusion criteria were as follows: hemorrhagic stroke, craniocerebral trauma, cardiogenic thrombosis and tumors. Controls matched the cases at the age and gender frequency were selected from the hospital's health care center during the same period. Individuals with autoimmune diseases, liver diseases, genetic diseases, blood disorders and tumors were excluded. The clinical data such as age, gender, hypertension, diabetes, smoking status, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A1 (Apo-A1), apolipoprotein B (Apo-B) and non-esterified fatty acid (NEFA) were collected from our medical records. The interval time was within 24 hours between IS onset and biochemical test.

SNPs selection

The selection criteria for SNP are as follows: (i) tagSNPs in lncRNA MALAT 1; (ii) in silico analysis predicted potentially functional SNPs in the promoter region of MALAT1; (iii) the frequency of minor allele > 5% in Chinese Han population. Finally, four SNPs of rs600231 A > G, rs1194338 C > A, rs4102217 G > C and rs591291 C > T were selected for further analysis.

Genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells by a salting-out method. About 3–5 ml blood samples was taken into ethylene diamine tetra acetic acid tubes from each subject before treatment. Genotyping was performed on an ABI 3500 Genetic Analyzer (Applied Biosystems, CA, USA) using the custom-by-design 48-Plex SNPscan kit (Genesky Biotechnologies Inc, Shanghai, China). Genotypic primers for rs600231, rs1194338, rs4102217and rs591291 were showed in Table 1. Meanwhile, about 10% of all samples were selected at random for Sanger sequencing and reached a 100% consistent rate.

Table 1

The primer sequences used for detecting four SNPs of the MALAT1 gene

SNPs	allele A1	allele A2	universal primer
rs600231	5'-TGAAACCCAGCAGACAGGACT-3'	5'-TGAAACCCAGCAGACAGGACC-3'	5'-GTCACTTCACAGAGAGCTGAGGGC-3'
rs1194338	5'-GGCTCCAGGGCCGTAGATCAA-3'	5'-GGCTCCAGGGCCGTAGATCAC-3'	5'-GGATCTCTCAGAAGCTTGTCTCTTGA-3'
rs4102217	5'-CCTGCTGCCTCCCTTCCTGTG-3'	5'-CCTGCTGCCTCCCTTCCTGTC-3'	5'-CAGCACTTCTGTCAGTCTCTCCAA-3'
rs591291	5'-CCCTCACCCCCGGGTCTGTG-3'	5'-CCCTCACCCCCGGGTCTGTA-3'	5'-GAACCTGTATCCATGGCTTGTTTTT-3'
SNPs single nucleotide polymorphisms.			

Statistical analysis

The Student's t-test was used to compare continuous data (Mean ± SD) such as clinical data between IS cases and controls. The chi-squared test was chosen to analyze Hardy-Weinberg equilibrium (HWE) and categorical data (proportions) such as sex, hypertension, diabetes mellitus and smoker data. Logistic regression was used to assess the risk of IS by odds ratios (OR), 95% confidence intervals (CIs) and P value after adjustment of age, gender, diabetes mellitus, hypertension, smoker, TC, TG, HDL-C, LDL-C, Apo-A1, Apo-B and NEFA. Linkage disequilibrium (LD) and haplotype analysis were carried out by SHEsis software (http://analysis.bio-x.cn/myAnalysis.php). The SPSS statistical software package version 20.0 (SPSS, Chicago, USA) was used for all of the statistical analysis. $P < 0.05$ was considered as significant statistically.

Results

Characteristics of the study population

The results are exhibited in Table 2. No significant difference was observed in distributions of age and gender between cases and controls. The frequencies of hypertension, diabetes mellitus and smoker in IS patients were obviously higher than those in controls ($P < 0.05$). In addition, IS patients displayed higher levels of TC, TG, LDL-C and Apo-B while lower levels of HDL-C, Apo-A1, and NEFA (all $P < 0.05$).

Table 2
Clinical characteristics of the study population

Variables	Controls, n = 320	IS patients, n = 316	P value
Age, years (Mean ± SD)	60.72 ± 10.77	62.23 ± 11.36	0.087
Gender (M / F)	204 / 116	216 / 100	0.220
Hypertension (%)	59 (18.4%)	126 (39.9)	< 0.001
Diabetes mellitus (%)	30 (9.4%)	50 (15.8%)	< 0.001
Smoker (%)	50 (15.6%)	98 (31.0%)	< 0.001
TCH (mmol/L)	4.19 ± 0.79	4.64 ± 1.16	< 0.001
TG (mmol/L)	1.37 ± 0.99	1.81 ± 1.31	< 0.001
HDL-C (mmol/L)	1.50 ± 0.31	1.13 ± 0.32	< 0.001
LDL -C (mmol/L)	2.39 ± 0.66	2.92 ± 0.98	< 0.001
Apo-A1(g/L)	1.73 ± 1.10	1.23 ± 0.26	< 0.001
Apo-B (g/L)	0.77 ± 0.31	1.00 ± 0.31	< 0.001
NEFA (mmol/L)	0.71 ± 0.30	0.53 ± 0.28	< 0.001

IS ischemic stroke, SD standard deviation, M male, F female, TC total cholesterol, TG triglyceride, HDL-C high density lipoprotein-cholesterol, LDL-C low density lipoprotein-cholesterol, Apo-A1 apolipoprotein A1, Apo-B apolipoprotein B, NEFA non-esterified fatty acid.

Association of MALAT1 polymorphisms with IS risk

The analysis of SNPs of MALAT1 for IS risk are revealed in Table 3. The genotype distributions in controls conformed to HWE ($P = 0.780$ for rs600231, $P = 0.858$ for rs1194338, $P = 0.569$ for rs4102217, $P = 0.582$ for rs591291). Among these SNPs of MALAT1, a decrease risk of IS was associated with the rs1194338 AC, AA and AC/AA genotype along with adjusted OR of 0.623, 0.474 and 0.596 respectively (AC vs. CC: 95% CI, 0.417–0.932, $P = 0.021$; AA vs. CC: 95% CI, 0.226–0.991, $P = 0.047$;

AC/AA vs. CC: 95% CI, 0.406–0.874, P = 0.008). Similarly, IS risk of carriers with A allele was reduced than C allele (AOR = 0.658, 95% CI, 0.487–0.890, P = 0.007). No significant association was found between other SNPs (rs600231, rs4102 217, rs591291) and IS risk.

Table 3
Association between the MALAT1 polymorphisms and risk of IS

SNPs	Controls n = 320(n%)	IS patient n = 316(n%)	AOR†(95%CI)	P† value
rs600231				
AA	117 (36.6)	120 (38.0)	1.000 (ref)	
AG	151 (47.2)	154 (48.7)	0.987 (0.651–1.496)	0.950
GG	52 (16.2)	42 (13.3)	0.778 (0.434–1.395)	0.399
Dominant			0.933 (0.629–1.386)	0.733
Recessive			1.276 (0.750–2.171)	0.369
A	385 (60.2)	394 (62.3)	1.000 (ref)	
G	255 (39.8)	238 (37.7)	0.906 (0.688–1.192)	0.481
rs1194338				
CC	154 (48.1)	188 (59.5)	1.000 (ref)	
AC	135 (42.2)	106 (33.5)	0.623 (0.417–0.932)	0.021
AA	31 (9.7)	22 (7.0)	0.474 (0.226–0.991)	0.047
Dominant			0.596 (0.406–0.874)	0.008
Recessive			1.721 (0.841–3.523)	0.137
C	443 (69.2)	482 (76.3)	1.000 (ref)	
A	197 (30.8)	150 (23.7)	0.658 (0.487–0.890)	0.007
rs4102217				
GG	243 (75.9)	237 (75.0)	1.000 (ref)	
CG	73 (22.8)	69 (21.8)	1.186 (0.753–1.868)	0.463
CC	4 (1.3)	10 (3.2)	2.322 (0.605–8.906)	0.219
Dominant			1.254 (0.807–1.947)	0.314
Recessive			0.452 (0.119–1.721)	0.244
G	559 (87.3)	543 (85.9)	1.000 (ref)	
C	81(12.7)	89 (14.1)	1.280 (0.869–1.886)	0.212
rs591291				
CC	123 (38.5)	129 (40.8)	1.000 (ref)	
CT	147 (45.9)	144 (45.6)	0.916 (0.607–1.384)	0.678
TT	50 (15.6)	43 (13.6)	0.752 (0.421–1.343)	0.336
Dominant			0.873 (0.592–1.289)	0.495

IS ischemic stroke, OR odds ratio, 95% CI, 95% confidence interval, † Adjusted by age, gender, hypertension, diabetes mellitus, smoker, TCH, TG, HDL-C, LDL-C, Apo-A1, Apo-B, NEFA.

SNPs	Controls n = 320(n%)	IS patient n = 316(n%)	AOR†(95%CI)	P† value
Recessive			1.268 (0.743–2.163)	0.384
C	393 (61.4)	402 (63.6)	1.000 (ref)	
T	247 (38.6)	230 (36.4)	0.877 (0.665–1.155)	0.350

IS ischemic stroke, OR odds ratio, 95% CI, 95% confidence interval, † Adjusted by age, gender, hypertension, diabetes mellitus, smoker, TCH, TG, HDL-C, LDL-C, Apo-A1, Apo-B, NEFA.

Haplotype analysis of the MALAT1 gene

To further estimated the association between MALAT1 polymorphism and IS risk, we performed Haplotype analysis. The analysis showed that the rs600231 was in strong linkage disequilibrium with rs591291 (D'= 0.94, r^2 = 0.83). As summarized in Table 4, possible four haplotypes were listed, rs600231-rs1194338-rs4102217-rs591 291 (A-C-G-C) haplotype had a 1.3-fold increase of IS risk (95% CI, 1.029–1.644, P = 0.027).

Table 4
Haplotype analysis of the MALAT1 polymorphisms with risk of IS

Haplotype	Controls (n %)	IS (n %)	OR (95%CI)	P value
ACGC	351 (54.9)	373 (59.1)	1.301 (1.029–1.644)	0.027
GAGT	91 (15.3)	86 (13.6)	0.903 (0.659–1.238)	0.527
GACT	71(11.1)	57 (9.1)	0.822 (0.569–1.187)	0.296
GCGT	61(9.6)	62 (9.9)	1.076 (0.742–1.561)	0.699

IS ischemic stroke, OR odds ratio, 95% CI 95% confidence interval. Only frequency greater than 1% is listed.

Multivariate logistic regression analysis

As shown in Table 5, the rs1194338 AC/AA affect the IS risk together with blood lipid index. The specific data were as follows: TC (OR = 1.607; 95%CI, 1.356–1.903), TG (OR = 1.482; 95%CI, 1.242–1.770), HDL-C (OR = 0.020; 95%CI, 0.010–0.040), LDL-C (OR = 2.181; 95%CI, 1.764–2.697), Apo-A1 (OR = 0.006; 95%CI, 0.002–0.013), Apo-B (OR = 23.315; 95%CI, 11.576–46.959), NEFA (OR = 0.092; 95%CI, 0.048–0.177) and rs1194338AC/AA (OR = 0.632; 95%CI, 0.461–0.865) (all $p < 0.05$).

Table 5
Logistic regression analysis for independent factors of IS susceptibility

Variables	B	OR (95%CI)	P value
TC	0.47	1.607 (1.356–1.903)	< 0.001
TG	0.39	1.482 (1.242–1.770)	< 0.001
HDL-C	-3.89	0.020 (0.010–0.040)	< 0.001
LDL-C	0.78	2.181 (1.764–2.697)	< 0.001
Apo-A1	-5.193	0.006 (0.002–0.013)	< 0.001
Apo-B	3.149	23.315 (11.576–46.959)	< 0.001
NEFA	-2.389	0.092 (0.048–0.177)	< 0.001
rs1194338AC/AA	-0.459	0.632 (0.461–0.865)	0.004

TC total cholesterol, TG triglyceride, HDL-C high density lipoprotein-cholesterol, LDL-C low density lipoprotein-cholesterol, Apo-A1 apolipoprotein A1, Apo-B apolipoprotein B, NEFA non-esterified fatty acid.

The SNPs of MALAT1 and blood lipid levels analysis

The association between MALAT1 polymorphism and lipid levels was analyzed in IS(Table 6). Unfortunately, no evidence of association was observed between SNPs of MALAT1 and clinical blood lipid levels ($P > 0.05$).

Table 6
Association between the MALAT1 SNPs and Blood lipid levels in IS

SNPs	TC, mmol/L	TG, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	Apo-A1, g/L	Apo-B, g/L	NEFA, mmol/L
rs600231							
AA	4.65 ± 1.20	1.79 ± 1.34	1.13 ± .33	2.94 ± 1.03	1.23 ± 0.26	1.00 ± 0.32	0.53 ± 0.28
AG/GG	4.64 ± 1.08	1.83 ± 1.27	1.12 ± .31	2.88 ± 0.90	1.23 ± 0.25	1.00 ± 0.30	0.55 ± 0.28
t	0.090	-0.245	0.384	0.462	-0.138	0.007	-0.620
p	0.928	0.806	0.701	0.645	0.890	0.995	0.535
rs1194338							
CC	4.68 ± 1.11	1.79 ± 1.38	1.14 ± 0.32	2.94 ± 0.97	1.23 ± 0.23	0.99 ± 0.30	0.52 ± 0.27
AC/AA	4.62 ± 1.19	1.82 ± 1.27	1.12 ± 0.32	2.90 ± 0.99	1.23 ± 0.28	1.01 ± 0.31	0.54 ± 0.29
t	0.478	-0.170	0.519	0.434	-0.032	-0.382	0.498
p	0.633	0.865	0.604	0.664	0.974	0.703	0.619
rs4102217							
GG	4.52 ± 1.21	1.72 ± 1.20	1.12 ± 0.30	2.80 ± 0.99	1.21 ± 0.26	0.98 ± 0.30	0.55 ± 0.29
CG/CC	4.68 ± 1.14	1.83 ± 1.35	1.13 ± 0.33	2.96 ± .98	1.24 ± 0.26	1.01 ± 0.31	0.53 ± 0.28
t	-1.079	-0.677	-0.374	-1.274	-0.642	-0.861	0.562
p	0.281	0.499	0.708	0.204	0.522	0.390	0.574
rs591291							
CC	4.64 ± 1.22	1.82 ± 1.40	1.13 ± 0.33	2.93 ± 1.05	1.23 ± 0.27	1.00 ± 0.32	0.53 ± 0.28
CT/TT	4.63 ± 1.07	1.78 ± 1.19	1.12 ± 0.31	2.89 ± 0.88	1.24 ± 0.25	1.00 ± 0.29	0.54 ± 0.28
t	0.072	0.262	0.124	0.460	-0.447	0.083	-0.550
p	0.943	0.794	0.902	0.646	0.655	0.934	0.583

TC total cholesterol, TG triglyceride, HDL-C high density lipoprotein-cholesterol, LDL-C low density lipoprotein-cholesterol, Apo-A1 apolipoprotein A1, Apo-B, apolipoprotein B, NEFA non-esterified fatty acid.

Bioinformatics analysis of MALAT1 rs1194338 with gene expression

GTEx data (https://www.gtexportal.org/home/) was used to identify correlations between SNPs and tissue-specific gene expression levels. Expression Quantitative Trait Loci (eQTL) showed the rs1194338 SNPs were associated with MALAT1 gene expression in single tissue (Fig. 1A), and AA genotype was higher expression in brain association tissues such as brain-hippocampus, brain-cerebellar hemisphere (Fig. 1B-C) ($P < 0.001$).

Discussion

In the current study, we analyzed the association between SNPs in the promoter of MALAT1 gene and IS risk. Significant differences were observed in the distribution of the rs1194338 AC/AA genotype and A allele between controls and cases. Further analysis shows that MALAT1 rs1194338 A allele, AA, AC genotype and the dominant model were associated with decreasing IS risk. Regression analysis revealed that rs1194338AC/AA was impact factors of IS together with lipid index

such as TC, TG, HDL - C, etc. In addition, results from Haplotype analysis showed that rs600231-rs1194338-rs4102217-rs591291 (A-C-G-C) haplotype had a 1.3-fold increase of IS risk. These findings implicate that analysis of rs1194338 may reveal the roles of MALAT1 in the etiology of IS in the future.

MALAT1, an 8.1 kb lncRNA, located on human chromosome 11q13. In 2003, Ji et al discovered firstly and named from lung cancer cells[25]. Subsequently, MALAT1 was reported to be associated with tumors cell proliferation, metastasis, survival, and recurrence[26]. New evidences show that MALAT1 was abundantly expressed in vascular endothelial cells and participated in processes of neuroprotection of IS by improvement of cognitive function, promotion of angiogenesis, inhibition of apoptosis and inflammation, regulation of autophagy, and protection of blood-brain barrier function[15–17, 27, 28]. The PI3K/AKT pathway have recently been implicated in cell proliferation, apoptosis, and survival in physiological and pathological conditions[29]. Study showed a neuroprotective role of early activation of PI3K in ischemic stroke[30]. The result from Yuan et al. invested that overexpression of MALAT1 decreased cell apoptosis by activating of PI3K/AKT pathway, eventually protect human cerebrovascular endothelial cells in OGD and reoxygenation condition[31]. The above indicates that the MALAT1 plays a critical role in ischemic stroke, and its high expression may contribute to the protection against brain injury.

The ischemic stroke is one of diseases threatening human health, which underlying mechanism are less well understood. Actually, increasing studies focused on SNPs of lncRNA involved in process of IS. For example, the rs2240183 C allele of lncRNA TUG1 was associated with a higher risk of IS possibly by binding to GATA-1 and elevating TUG1 levels [20], the ANRIL rs2383207 increased the risk of IS by 1.52-fold under the recessive mode [21], the rs217727 TT and rs4929984 AA in the H19 increase the risk of IS, with adjusted OR 4.288, 3.020, respectively [22]. Those provide a new perspective on the genetic mechanism of IS. Given above, we hypothesized that the MALAT1 gene polymorphisms are associated with IS risk. Our results supported the above assumption. As shown in Table 3, case-control studies indicated the rs1194338 A allele, AC and AA genotype of MALAT1 contributed to the decrease of IS susceptibility, and A-C-A-G haplotype increased IS risk (Table 4). logistic regression also validated effect of the rs1194338 AC/AA for IS risk (Table 5).

The rs1194338, a functional site, located in the promoter region of the MALAT1. Recently, several studies indicated the relationship between rs1194338 variant and human diseases. In hepatocellular carcinoma (HCC), female patients and patients with a smoking habit who carried the CA + AA genotype of rs1194338 had a lower risk of developing vascular invasion and a high Child-Pugh grade, respectively[32]. This suggested there was an interactive function between rs1194338 and the environment, whether it interacts with the environment in IS remains to be further explored. In colorectal cancer, previous study found carriers with AA and AC genotype of the rs1194338 were lower risk than CC genotype, and the conclusion from Li's study showed no statistically significant difference in expression levels of MALAT1 between CC and AA genotype at rs1194338[33, 34]. However, data from the GTEx database showed rs1194338 SNP had a difference in expression of MALAT1. Particularly, the rs1194338 SNP AA indicated a significant increase of expression compared to the CC genotype in brain brain-hippocampus and cerebellar hemisphere tissues ($P < 0.001$) (Fig. 1). Based on the above background, we hypothesized that the rs1194338 AC/AA genotype may increase the expression levels of MALAT1, which activating related pathways such as PI3K/AKT, thereby reducing the risk of IS. Further studies are needed to investigate the correlation between the rs1194338 SNP and MALAT1 expression and the precise mechanism of rs1194338 SNP in IS.

At present, studies on rs600231 A > G variant with disease have not been reported, but rs4102217 and rs591291 SNPs were evaluated in rheumatoid arthritis (RA) and HCC. Zhang et al. indicated rs4102217 and rs591291 SNPs were not associated with RA susceptibility[35]. Studies of association with HCC have shown rs4102217 had a 1.32-fold risk in the dominant model, and rs591291 highlighted better prognosis in female and HBV negative subgroups, but association between MALAT1 haplotype (rs4102217-rs591291-rs11227209- rs619586) and HCC risk were not found[22]. In our study, we found that A-C-A-G (rs600231-rs1194338- rs4102217-rs591291) haplotype had a 1.3-fold IS risk (Table 4) although SNPs (rs600231, rs4102217, rs591291) did not correlate with IS susceptibility. It is well known that alteration in blood lipid level is one of the relevant risk factor in atherosclerotic plaques formation, which cause easily hypoxia and possibly lead to injury in downstream tissues such as ischemic stroke[36]. According to the report, MALAT1 involved in lipid metabolism[37]. Thus, we further analyzed the

relationship between the SNPs of MALAT1 and blood lipids. Unfortunately, we did not observe a relationship between the four SNPs of the MALAT1 and lipid levels. These founding would help improve our understanding of the roles of MALAT1 genetic variants in the pathogenesis of IS.

Our study is not without limitations. Firstly, a relatively small sample may limit the statistical analysis of our research. Secondly, there are distribution differences in polymorphisms of the same locus among different races according to the 1000 Genomes Project Data. The population we studied came from the southwest of China. It was a hospital-based case-control study, which may have a choice bias. Therefore, larger sample sizes from other medical centers among different races and ethnic groups are needed to further confirm the role of MALAT1 SNPs in IS susceptibility. Finally, the functional correlation of MALAT1 SNPs in IS is very interesting, but it is not clear, both RNA and DNA should be collected simultaneously from the same samples to further verify the effect of SNPs on MALAT1 expression.

Conclusions

In conclusion, it is just beginning that functional analysis of lncRNA MALAT1 for IS risk. Our study provides a link between rs1194338 of MALAT1 promoter region and IS risk, which help to identify the potential molecular mechanisms. In the future, large- scale samples study can be performed among different populations.

Abbreviations

Apo-A1: Apolipoprotein A1; ApoB: Apolipoprotein B; CIs: Confidence intervals; HCC:hepatocellular carcinoma; HDL-C:high-density lipoprotein cholesterol; HWE:Hardy-Weinberg equilibrium;IS: ischemic stroke; LD:linkage disequilibrium; LDL-C:low-density lipoprotein cholesterol; lncRNAs:long non-coding RNAs; MALAT1:Metastasis-associated lung adenocarcinoma transcript-1; NEFA:non-esterified fatty acid; OGD:oxygen-glucose deprivation; SNP:single nucleotide polymorphism; TC:total cholesterol, TG:triglyceride.

Declarations

Acknowledgements

We are deeply grateful to all participants of this study.

Funding

This work was supported by the Natural Science Foundation of China, Grant/Award Number: 81560552; Natural Science Foundation of Guangxi, China, Grant/Award Number: 2018GXNSFAA138120; Key Research Projects of Guangxi, China, Grant/Award Number: 2018AB58018.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Authors' contributions

Ye-Sheng Wei guided and revised manuscript. Yan Wang and Xi-Xi Gu participated in experiment and wrote the manuscript. Hua-Tuo Huang helped to perform experiments. Chun-Hong Liu performed the statistical analysis and figures. All authors reviewed the manuscript.

Ethics approval and consent to participate

The procedure was reviewed and approved by the Institutional Ethical Committee of the Affiliated Hospital of Youjiang Medical University for Nationalities, in accordance with the principles of the Helsinki Declaration. Each subject signed

informed consent and agreed to make the data public.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing.

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Figures

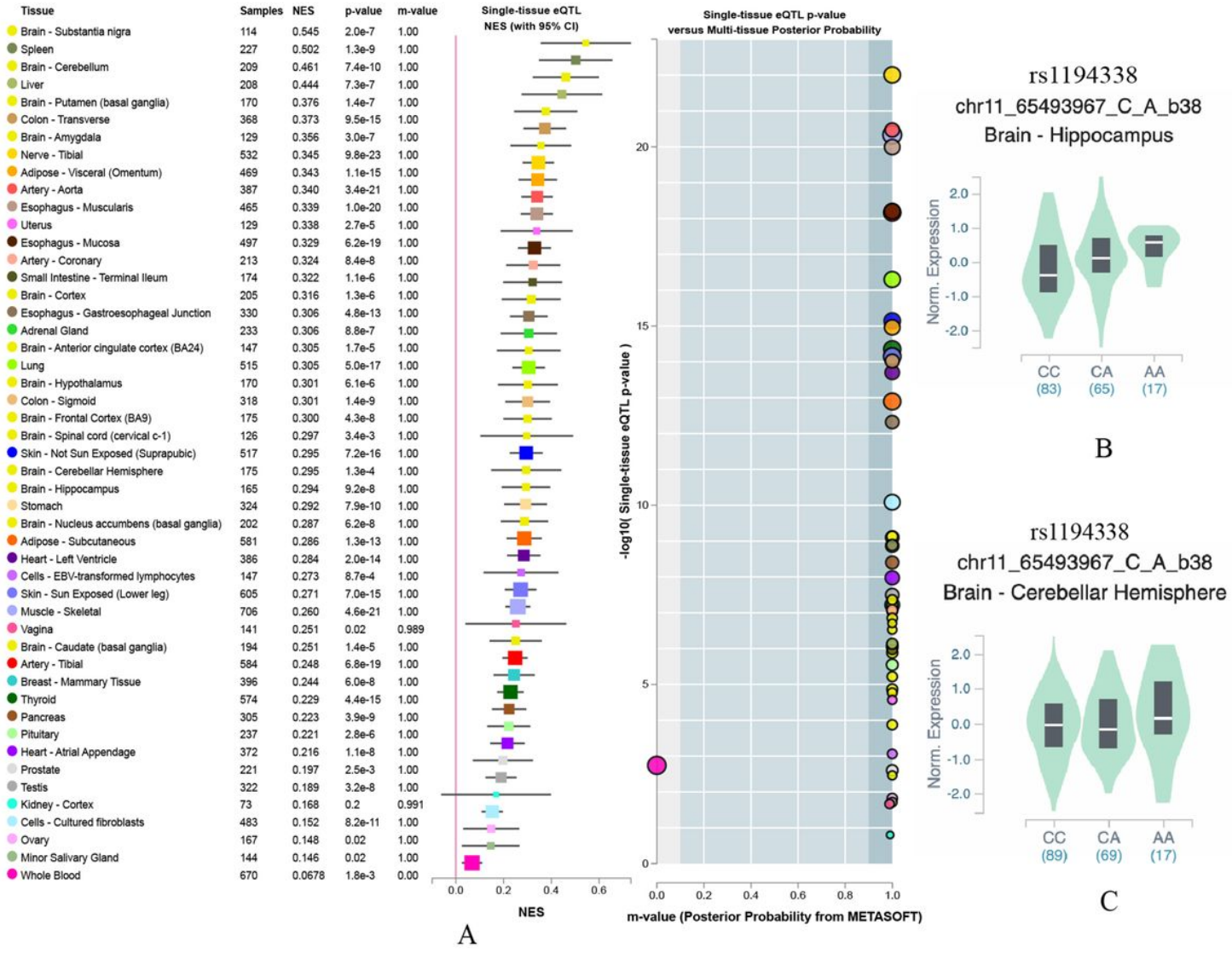

Figure 1

Expression quantitative trait loci (eQTL) anlysis of rs1194338 Expression Quantitative Trait Loci analysis of rs1194338 with gene expression in single tissue (A), brain-hippocampus (B), and brain-cerebellar hemisphere (C).