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LINC01492 is associated with susceptibility to large atherosclerotic stroke and levels of inflammatory factors

Jianyu Liu[1,2#], Da Liu[3#], Xing Guo[4], Li Zhou[5], Zhiyao Xu[6], Hua Liu[1]

[1]Department of Neurology, The Affiliated Hospital of Southwest Jiaotong University, The Third People's Hospital of Chengdu, Chengdu, [2]Medical Research Center, The Affiliated Hospital of Southwest Jiaotong University, The Third People's Hospital of Chengdu, Chengdu, [3]Department of Clinical Medicine, North Sichuan Medical College, Nanchong, [4]Institute of Biomedical Engineering, College of Medicine, Southwest Jiaotong University, Chengdu, [5]Department of Neurology, The First People's Hospital of Neijiang, Neijiang, [6]College of Medicine, Southwest Jiaotong University, Chengdu, Sichuan, China

[#]Jianyu Liu and Da Liu contributed equally to this study

Address for correspondence:
Dr. Hua Liu, Department of Neurology, The Affiliated Hospital of Southwest Jiaotong University, The Third People's Hospital of Chengdu, Chengdu, Sichuan, China.
E-mail: hxliumedidoctor@163.com

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Abstract:

BACKGROUND: LINC01492 polymorphism has been implicated in the susceptibility of large artery atherosclerotic (LAA) stroke; however, a dearth of systematic research exists regarding this association in the Asian population at present.

SUBJECTS AND METHODS: This study enrolled Han Chinese patients with LAA stroke (n = 428) and age- and sex-matched controls (n = 434). We employed dominant, recessive, and codominant genetic models to analyze the distribution of alleles and genotypes for 14 tag single nucleotide polymorphisms (SNPs) in LINC01492. Furthermore, we quantified the transcript levels of LINC01492 as well as concentrations of inflammatory factors (interleukin [IL]-1β, IL-6, IL-8, IL-10, IL-18, tumor necrosis factor alpha, and CCL18). In addition, we explored the association between these SNPs and levels of inflammatory factors.

RESULTS: The rs10990654 AA genotype of LINC01492 was markedly associated with a heightened risk of LAA stroke (odds ratio [OR] = 6.403, 95% confidence interval [CI] = 1.180–34.734, P = 0.031). Conversely, both the GG genotype of rs10990654 (OR = 0.614, 95% CI = 0.384–0.980, P = 0.041) and the GG genotype of rs16922693 (OR = 0.518, 95% CI = 0.313–0.857, P = 0.010) were identified as being linked to a reduced risk in this study population. In addition, the transcription level of LINC01492 was markedly reduced in patients compared to controls. Furthermore, significant variations were observed in IL-10 and IL-18 levels across genotypes at LINC01492 polymorphism loci among patients.

CONCLUSIONS: The genetic polymorphisms and transcript levels of LINC01492 exhibit an association with susceptibility to LAA stroke, and the available evidence suggests that this association may be mediated through IL-10 and IL-18.

TRIAL REGISTRATION: The trial was registered with the Chinese Clinical Trial Registry (www.chictr.org.cn) under trial registration number ChiCTR2000032684.

Keywords:

Genetic risk factors, inflammation, large artery atherosclerotic stroke, LINC01492, single nucleotide polymorphisms

Introduction

Long intergenic noncoding RNAs (lincRNAs) are autonomously transcribed, nonoverlapping RNA molecules spanning over 200 nucleotides and lacking coding potential. They exert a broad range of functions in modulating gene expression, directly influencing nuclear architecture and sequestering intracellular molecules or facilitating their activity, as well as indirectly impacting transcription or translation processes. The roles of lincRNAs in disease and development are pivotal, rendering them potential biomarkers and therapeutic targets with significant implications.[1]

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Numerous lincRNAs exhibit an enrichment of disease-associated genetic polymorphism loci. Refining the impact of genetic factors on stroke could more precisely delineate causal pathways, unveil novel therapeutic targets, and enhance diagnostic and prognostic options.[2] The heritability of large artery atherosclerotic (LAA) stroke surpasses that of other subtypes, thereby emphasizing the significance of identifying genetic risk factors specific to LAA stroke. The investigation of the correlation between lincRNA polymorphism and LAA holds significant value.[3]

The LINC01492 gene is situated on the long arm of chromosome 9, and a study involving 521,612 individuals demonstrated a notable correlation between its polymorphism and susceptibility to LAA stroke. The association between LINC01492 and LAA stroke sensing is highly specific, as it only showed an association with LAA stroke and not with other isoforms.[4] The prevalence of ischemic stroke (IS) is 62.4% among all strokes, with LAA stroke being the most predominant subtype.[3,5] Therefore, elucidating the correlation between LINC01492 and LAA stroke holds significant implications.[4]

Emerging evidence suggests that lincRNAs play critical roles in the development of atherosclerosis, a major underlying cause of LAA stroke.[6] LAA stroke is primarily driven by the accumulation of atherosclerotic plaques in large arteries, which is mediated by endothelial dysfunction, immune responses, and chronic inflammation.[7] Notably, inflammation plays a central role in the pathogenesis of atherosclerosis, thrombosis, and cerebrovascular diseases, all of which are fundamental mechanisms in LAA stroke development.[8]

Among the inflammatory mediators, interleukins (ILs) are critical in regulating the immune response during LAA stroke. For instance, IL-10, which binds to the IL-10 receptor (IL-10R), suppresses inflammation and limits apoptosis, playing a protective role in ischemic brain injury.[9,10] In addition, IL-18 is recognized as a pro-inflammatory cytokine that has been linked to stroke-induced inflammation, with the initial serum level of IL-18 serving as a prognostic marker for stroke outcomes.[11] Despite these insights into inflammatory processes, no studies to date have directly examined the relationship between LINC01492 polymorphisms, inflammation, and LAA stroke susceptibility.

In this study, we selected 14 tag single nucleotide polymorphisms (SNPs) from LINC01492 and conducted a comprehensive investigation into the association between these genetic loci and susceptibility to LAA stroke, as well as their impact on levels of relevant inflammatory factors.

Subjects and Methods

Study populations

We consecutively enrolled a cohort of individuals who experienced their first-ever stroke caused by LAA stroke from the Neurology Department of The Third People's Hospital of Chengdu. The diagnosis of LAA stroke was based on the Chinese IS subclassification.[12] To serve as controls, we randomly selected healthy adults from the physical examination center at the identical hospital during the same recruitment period, matching them with cases based on age, sex, and ethnic group. Controls were confirmed to be free of any vascular or neurological diseases through questionnaires, medical history assessment, and clinical examination. Detailed demographic information and risk factors including age, sex, hypertension, diabetes, heart diseases, hyperlipidemia, smoking status, drinking habits, overweight, carotid stenosis, and hyperhomocysteinemia (HHCY) levels were meticulously recorded while ensuring confidentiality by two department staff members.

Clinical characteristics

The participants' demographic characteristics, potential factors that may increase the risk of stroke, and their medical history were collected for analysis. Cigarette smoking was defined as the regular consumption of a minimum of one cigarette per day for at least period of 1 year.[13] Alcohol consumption was considered present if the individual had consumed alcohol at least 12 times in the past year.[13] Hypertension diagnosis followed the guidelines provided by both the World Health Organization (WHO) and the International Society of Hypertension, which define hypertension as having a blood pressure of 140/90 mmHg or higher or being on antihypertensive medications.[14] Diabetes mellitus diagnosis relied on elevated fasting plasma glucose levels ($\geq$7.0 mM) or documented use of oral hypoglycemic agents or insulin treatment, following WHO criteria.[15] Dyslipidemia was defined based on the 2016 Chinese guideline for managing dyslipidemia in adults.[16] Being overweight is determined by having a body mass index equivalent to or >24 kg/m^2 based on Chinese criteria.[17] HHCY is defined as having a plasma total homocysteine concentration higher than 10 μmol/L.[18] Carotid stenosis is diagnosed when there is more than 50% blockage observed through angiography imaging.[19]

Single nucleotide polymorphisms selection and genotyping

We obtained genotypes for SNPs in LINC01492 that represent individuals of Han Chinese descent from the HapMap database (phase II, 8 November, on NCBI B36 assembly, dbSNP b126). To ensure a lower similarity score, we meticulously handpicked

14 tagSNPs (rs7853468, rs78773706, rs79997970, rs10739871, rs10820385, rs10990616, rs10990618, rs10990647, rs10990654, rs16922693, rs373637, rs384867, rs4743634, and rs7044631) based on their strong linkage disequilibrium (LD) ($r^2 > 0.8$) and a minor allele frequency exceeding 0.05 within the Chinese Han population.

A total volume of 5 mL venous blood was obtained from the antecubital vein in the morning following an overnight period of fasting. The blood was collected using EDTA (disodium salt, 50 mmol/L) tubes and subsequently stored at a temperature of −80°C until further analysis. Genomic DNA extraction was performed on peripheral blood samples using the Tiangen Genomic DNA Extraction Kit. The genotypes of selected SNPs were determined utilizing SNPscan technology provided by Genesky. In brief, SNP loci allele identification relied on the high specificity of the ligase ligation reaction. Nonspecific sequences were introduced to the end of the ligation probe, followed by ligase addition reaction to generate ligated products with varying lengths. These products were then amplified through polymerase chain reaction (PCR) employing fluorescence-labeled universal primers and subsequent separation through fluorescence capillary electrophoresis [Supplementary Table 1]. Ultimately, analysis of electrophoretic profiles enabled the determination of genotypes for each SNP locus.

Quantitative real-time polymerase chain reaction

We conducted a random selection of LAA stroke patients and control samples to evaluate the transcript levels of LINC01492. Peripheral blood was used for total RNA isolation, employing the TRNzol Universal Total RNA Isolation Kit (Tiangen, Beijing, China). The quality assessment of total RNA was performed using agarose gel electrophoresis. For reverse transcription, 1 μg of total RNA was subjected to TransScript® Uni All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (Transgen, Beijing, China). Before PCR amplification, the resulting cDNA was diluted by a factor of 10. A negative control without reverse transcriptase was included. SYBR green real-time PCR (Yeasen, Shanghai, China) was utilized for measuring mRNA levels. Primer sequences are given in Supplementary Table 2.

Inflammation-related factors assay

The plasma samples were collected using EDTA as an anticoagulant and subsequently centrifuged at 1000 × *g* for 15 min at 4°C. The levels of tumor necrosis factor-alpha (TNF-α), CCL18, IL-1β, IL-6, IL-8, IL-10, and CCL-18 were quantified by flow cytometry based on the manufacturer's instructions (Nuohebio, Chengdu, China).

Statistical analysis

All statistical analyses were conducted utilizing SPSS statistics software version 21.0 (IBM, Armonk, USA). The Chi-square test was employed to evaluate the proportions of clinical and environmental variables, as well as Hardy–Weinberg equilibrium (HWE). Subsequent genetic association analyses were conducted utilizing three genetic models: dominant, recessive, and allelic comparison. Normally distributed continuous data were compared using a Student's *t*-test and expressed as mean ± standard deviation. To address type I errors, the Benjamini–Hochberg method of false discovery rate correction was applied. Gene–gene interactions were examined utilizing the GMDR beta v0.9 software package, which identifies the most optimal model combination based on multiple genes and behavioral indicators through factor dimensionality reduction principles. The significance level for determining meaningful models was set at $P < 0.05$; higher testing balance accuracy indicates better model performance; and closer cross-validation consistency values to 10 indicate stronger results. Multivariable logistic regression analysis was used to investigate the impact of high-risk interactive genotypes on functional outcomes while adjusting for relevant baseline variables identified in univariate analysis (enter approach with probability of entry $P < 0.2$). $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics of the subjects

In total, 434 patients diagnosed with LAA stroke and 428 individuals without the condition were included. The baseline clinical and demographic characteristics can be found in Table 1. Multifactor logistic regression analysis indicated notable distinctions in conventional risk factors for LAA stroke, including sex ($P = 0.025$), hypertension ($P < 0.001$), diabetes ($P < 0.001$), heart diseases ($P = 0.007$), smoking status ($P = 0.001$), and carotid stenosis ($P = 0.030$).

Rs10990654 and Rs16922693 are associated with susceptibility to large artery atherosclerotic stroke

The frequency distribution of the fourteen variants in the study population adheres to HWE ($P > 0.05$), indicating that the gene frequencies observed are representative of those found in the general population [Supplementary Table 3].

A multivariate regression analysis was conducted, incorporating age, sex, hypertension, diabetes, heart disease, smoking status, drinking habits, overweight, carotid stenosis, and HHCY as covariates. Our findings indicate a significant association between rs10990654 and rs16922693 with susceptibility to LAA stroke. Specifically, individuals with GG genotypes of rs10990654 (odds ratio [OR] = 0.614, 95% confidence interval [CI] =

Table 1: Baseline characteristics of the study population

Characteristics	LAA stroke (*n*=428), *n* (%)	Controls (*n*=434), *n* (%)	Unadjusted		Adjusted*	
			OR (95% CI)	*P*	OR (95% CI)	*P*
Age (years)	63.33±10.54	58.45±11.71		<0.001		
≥60	282 (65.9)	233 (53.6)	1.666 (1.266–2.193)	<0.001	0.855 (0.616–1.188)	0.351
<60	146 (34.1)	201 (46.3)				
Sex						
Male	274 (64.0)	189 (43.5)	2.306 (1.754–3.033)	<0.001	1.511 (1.053–2.170)	0.025
Female	154 (36.0)	245 (56.5)				
Hypertension						
Yes	321 (75.0)	175 (40.3)	4.440 (3.319–5.939)	<0.001	2.903 (2.089–4.035)	<0.001
No	107 (25.0)	259 (59.7)				
Diabetes						
Yes	180 (42.1)	67 (15.4)	3.976 (2.877–5.494)	<0.001	2.662 (1.862–3.805)	<0.001
No	248 (57.9)	367 (84.6)				
Heart diseases						
Yes	47 (11.0)	18 (4.1)	2.851 (1.627–4.995)	<0.001	2.364 (1.262–4.430)	0.007
No	381 (89.0)	416 (95.9)				
Hyperlipidemia						
Yes	288 (67.3)	276 (63.6)	1.178 (0.889–1.560)	0.254	1.038 (0.751–1.436)	0.820
No	140 (32.7)	158 (36.4)				
Smoking						
Yes	160 (37.4)	65 (15.0)	3.389 (2.440–4.707)	<0.001	2.338 (1.432–3.819)	0.001
No	268 (62.6)	369 (85.0)				
Drinking						
Yes	129 (30.1)	56 (12.9)	2.912 (2.055–4.126)	<0.001	1.093 (0.659–1.813)	0.730
No	299 (69.9)	378 (87.1)				
Overweight						
Yes	238 (55.6)	186 (42.9)	1.670 (1.276–2.186)	<0.001	1.336 (0.977–1.827)	0.069
No	190 (44.4)	248 (57.1)				
HHCY						
Yes	282 (65.9)	227 (52.3)	1.761 (1.338–2.318)	<0.001	1.029 (0.739–1.432)	0.867
No	146 (34.1)	207 (47.7)				
Carotid stenosis						
Yes	257 (60.0)	165 (38.0)	2.450 (1.863–3.222)	<0.001	1.423 (1.035–1.957)	0.030
No	171 (40.0)	269 (62.0)				

*Adjusted for age, sex, hypertension, diabetes, heart diseases, hyperlipidemia, smoking status, drinking habits, overweightness, carotid stenosis and HHCY.
LAA: Large artery atherosclerotic, HHCY: Hyperhomocysteinemia, OR: Odd ratio, CI: Confidence interval

0.384–0.980, $P = 0.041$) and rs16922693 (OR = 0.518, 95% CI = 0.313–0.057, $P = 0.010$) exhibit a reduced risk of LAA stroke; conversely, the AA genotype of rs10990654 is associated with an increased risk (OR = 6.403, 95% CI = 1.180–34.734, $P = 0.031$) [Table 2]. In addition, the transcription level of LINC01492 was revealed to be significantly diminished in patients compared to healthy controls [Figure 1].

Linkage disequilibrium analysis and haplotype block structure

As illustrated in Figure 2, the LD analysis revealed the presence of two blocks within the set of 14 tagSNPs. To ensure accuracy and minimize potential false positives, haplotypes with frequencies below 0.01 were excluded from further analysis. Following adjustment for various factors including age, sex, hypertension, diabetes, heart diseases, smoking status, drinking habits, overweightness, carotid stenosis, and HHCY, no significant association was found between any of the identified haplotypes and the risk of LAA stroke [Table 3].

Single nucleotide polymorphism-single nucleotide polymorphism interactions

Using the GMDR model, we conducted a comprehensive analysis to explore potential gene-gene interactions among the 14 tag SNPs in LINC01492; however, our findings did not reveal any statistically significant interactions between these genetic variants [Table 4].

LINC01492 polymorphism is associated with inflammation

Research has substantiated the influence of inflammatory factor levels on the development of LAA stroke.[20] The levels of TNF-α, CCL18, IL-1β, IL-6, IL-8, IL-10, and CCL-18 were also investigated in this study. Consistent

Table 2: Association of the LINC01492 polymorphism with risk of large artery atherosclerotic stroke

SNP	Genetic models	Unadjusted		Adjusted*	
		OR (95% CI)	*P*	OR (95% CI)	*P*
rs7853468	Dominant: CC/(TT+CT)	0.890 (0.669–1.183)	0.422	0.964 (0.694–1.339)	0.826
	Recessive: TT/(CC+CT)	0.788 (0.548–1.132)	0.196	0.732 (0.484–1.108)	0.140
	Codominant: T/C	0.986 (0.814–1.194)	0.885		
rs78773706	Dominant: AA/(GG+AG)	0.981 (0.730–1.318)	0.897	0.997 (0.711–1.399)	0.987
	Recessive: GG/(AA+AG)	1.427 (0.449–4.530)	0.545	1.658 (0.438–6.279)	0.457
	Codominant: G/A	1.035 (0.794–1.349)	0.798		
rs79997970	Dominant: GG/(CC+GC)	0.981 (0.723–1.333)	0.905	0.924 (0.651–1.312)	0.659
	Recessive: CC/(GG+GC)	0.843 (0.255–2.784)	0.779	0.522 (0.142–1.914)	0.327
	Codominant: C/G	1.006 (0.763–1.327)	0.966		
rs10739871	Dominant: CC/(AA+CA)	0.916 (0.687–1.222)	0.551	0.917 (0.658–1.278)	0.609
	Recessive: AA/(CC+CA)	0.971 (0.688–1.370)	0.868	1.056 (0.712–1.568)	0.785
	Codominant: A/C	1.030 (0.851–1.246)	0.762		
rs10820385	Dominant: CC/(GG+GC)	0.933 (0.683–1.275)	0.665	1.015 (0.706–1.458)	0.936
	Recessive: GG/(CC+GC)	1.014 (0.325–3.170)	0.981	1.178 (0.322–4.303)	0.805
	Codominant: G/C	1.059 (0.798–1.406)	0.690		
rs10990616	Dominant: CC/(TT+CT)	1.033 (0.787–1.357)	0.813	0.953 (0.698–1.303)	0.764
	Recessive: TT/(CC+CT)	0.862 (0.579–1.283)	0.465	0.924 (0.587–1.454)	0.732
	Codominant: T/C	0.948 (0.779–1.154)	0.595		
rs10990618	Dominant: GG/(AA+AG)	1.061 (0.774–1.455)	0.712	0.916 (0.634–1.323)	0.641
	Recessive: AA/(GG+AG)	1.536 (0.622–3.796)	0.349	2.220 (0.766–6.433)	0.142
	Codominant: A/G	0.995 (0.751–1.319)	0.974		
rs10990647	Dominant: GG/(AA+AG)	0.896 (0.684–1.174)	0.427	0.814 (0.595–1.112)	0.196
	Recessive: AA/(GG+AG)	0.876 (0.474–1.620)	0.673	0.927 (0.457–1.882)	0.834
	Codominant: A/G	1.059 (0.847–1.322)	0.616		
rs10990654	Dominant: GG/(AA+AG)	0.597 (0.398–0.896)	0.012	0.614 (0.384–0.980)	0.041
	Recessive: AA/(GG+AG)	4.640 (0.997–21.600)	0.026	6.403 (1.180–34.734)	0.031
	Codominant: A/G	1.766 (1.210–2.578)	0.003		
rs16922693	Dominant: CC/(GG+GC)	0.949 (0.724–1.244)	0.706	0.977 (0.716–1.334)	0.884
	Recessive: GG/(CC+GC)	0.666 (0.428–1.035)	0.069	0.518 (0.313–0.857)	0.010
	Codominant: G/C	0.945 (0.775–1.154)	0.580		
rs373637	Dominant: TT/(CC+CT)	0.972 (0.736–1.284)	0.840	0.859 (0.622–1.187)	0.358
	Recessive: CC/(TT+CT)	0.866 (0.598–1.254)	0.446	0.982 (0.641–1.503)	0.932
	Codominant: C/T	0.975 (0.804–1.182)	0.797		
rs384867	Dominant: CC/(TT+CT)	0.950 (0.717–1.259)	0.721	0.865 (0.625–1.196)	0.380
	Recessive: TT/(CC+CT)	0.868 (0.601–1.254)	0.450	1.017 (0.666–1.552)	0.939
	Codominant: T/C	0.985 (0.813–1.194)	0.879		
rs4743634	Dominant: TT/(CC+CT)	1.113 (0.836–1.483)	0.462	1.033 (0.743–1.438)	0.846
	Recessive: CC/(TT+CT)	0.875 (0.411–1.862)	0.729	0.964 (0.409–2.269)	0.933
	Codominant: C/T	0.909 (0.710–1.165)	0.453		
rs7044631	Dominant: GG/(AA+AG)	1.050 (0.781–1.413)	0.745	0.972 (0.688–1.372)	0.870
	Recessive: AA/(GG+AG)	0.724 (0.350–1.497)	0.380	0.912 (0.401–2.074)	0.826
	Codominant: A/G	0.924 (0.714–1.196)	0.550		

*Adjusted for age, sex, hypertension, diabetes, heart diseases, smoking status, drinking habits, overweightness, carotid stenosis and HHCY. To examine the connections between SNPs and LAA stroke risk, we employed three genetic models including dominant, recessive, and codominant. The LINC01492 SNPs consist of two variations: A (more common variation) and a (less common variation). These three models can be described as follows: The dominant model (comparing AA with aa+aA), the recessive model (comparing aa with aA+AA), and the codominant model (comparing an allele with A allele). SNP: Single nucleotide polymorphism, LAA: Large artery atherosclerotic, OR: Odd ratio, CI: Confidence interval

with previous research, our finding demonstrates significantly elevated levels of IL-10 and IL-18 in patients compared to the controls within this cohort (data not shown). Based on this, we conducted an analysis to examine the correlation between LINC01492 polymorphisms and the levels of IL-10 and IL-18 in both controls and patients. The statistics excluded certain genotypes due to their low frequency within the population. The results demonstrated significant differences in IL-10 levels among different genotypes of rs10990616, whereas distinct variations were observed in IL-18 levels among different genotypes of rs384867, rs7044631, rs78773796, and rs79997970 in patients. Furthermore, the controls did not exhibit any noteworthy disparities in the genotypes of these polymorphic loci [Figure 3].

Discussion

Our findings demonstrate that sex, hypertension, diabetes, heart disease, smoking, and carotid artery stenosis are significant risk factors for LAA stroke in the Chinese cohort of this study. Furthermore, we have made the novel discovery that there is a significant association between the transcript level of LINC01492 and the polymorphisms rs10990654 and rs16922693 with respect to susceptibility to LAA stroke. In addition, significant variations were observed in the levels of IL-10 or IL-18 among different genotypes of LINC01492 genetic polymorphism loci. The results indicate that genetic variations in LINC01492 are associated with the development of LAA stroke. The observed alterations in IL-10 and IL-18 levels point to a possible involvement of inflammatory pathways.

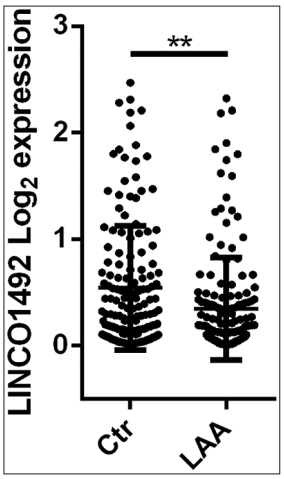

Figure 1: Transcription levels of LINC01492 in controls and large artery atherosclerotic stroke patients. Ctr: Controls, LAA: Large artery atherosclerotic stroke patients. Values are shown as means ± standard deviation, $^{**}P < 0.01$

The primary function of lincRNAs does not involve encoding effector proteins; however, they can still exert an impact on organismal function through diverse pathways. The transcription of target genes can be regulated by lincRNAs through the modulation of chromatin topology, thereby establishing a regulatory mechanism for the *HOXA* gene mediated by lincRNA HOTTIP.[21] The initiation of X-chromosome inactivation and the spreading of Xist rely on the interaction between EZH2, the catalytic subunit of PRC2, and RepA, a lincRNA located within the Xist locus that acts as a scaffolding and modulating factor.[22] The lincRNA Gas5 imitates the glucocorticoid response element by creating a dual-stranded configuration that attaches to the DNA-binding region of the glucocorticoid receptor. This interaction hinders the ability of the glucocorticoid receptor to bind its intended genes and initiate their transcription.[23] In addition, lincRNA can exert its function through the regulation of neighboring

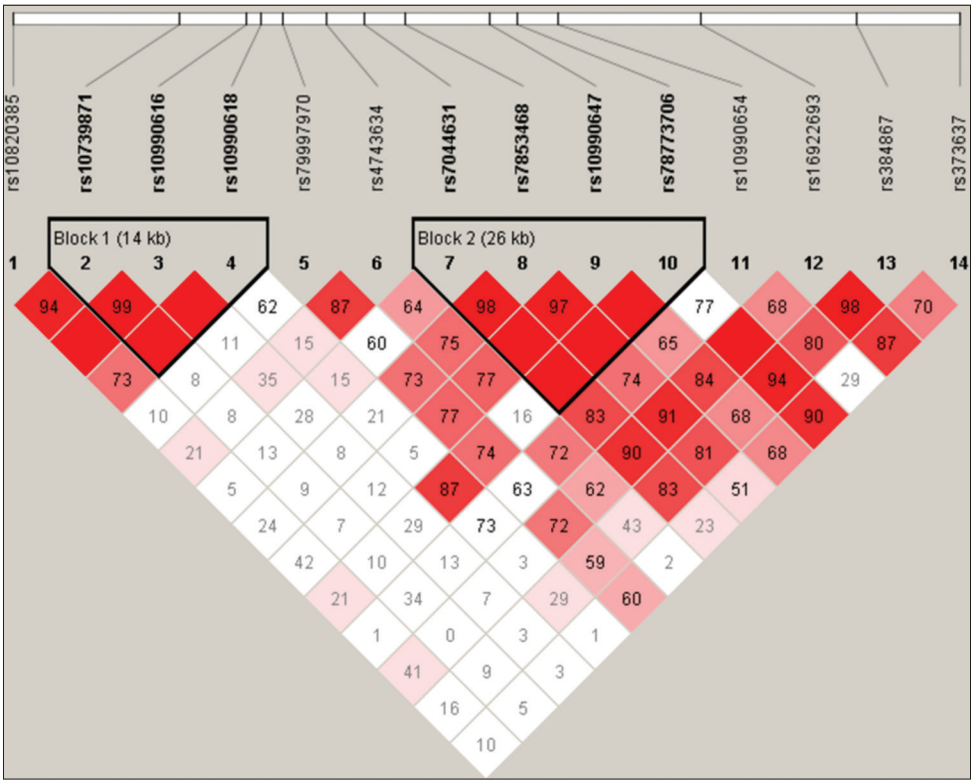

Figure 2: Linkage disequilibrium and correlation coefficients (r^2) among sixteen selected single nucleotide polymorphisms of the LINC01492 gene. This figure was generated using our sample population. The color gradient ranges from white to red, indicating increasing degrees of linkage. The values within the boxes represent D' values, with higher values indicating a stronger linkage between loci

Table 3: Association between haplotype and the risk of large artery atherosclerotic stroke

HapMap block	Haplotype*	Cases frequency	Controls frequency	Adjusted OR (95% CI)	*P***
Block 1	CTG	0.3572	0.371	1.00	
	ACG	0.3093	0.303	0.99 (0.76–1.29)	0.94
	CCG	0.2036	0.197	0.94 (0.69–1.28)	0.68
	ACA	0.1285	0.129	1.13 (0.79–1.60)	0.5
Block 2	GTGA	0.4171	0.4138	1.00	/
	GCAA	0.237	0.2256	1.15 (0.86–1.54)	0.35
	ACGA	0.1518	0.1623	1.04 (0.76–1.43)	0.79
	GCGG	0.1482	0.1469	1.06 (0.75–1.49)	0.75
	GCGA	0.0411	0.0441	0.80 (0.47–1.38)	0.42

*Haplotypes with frequency <1% were omitted, **Adjusted for age, sex, hypertension, diabetes, heart diseases, smoking status, drinking habits, overweightness, carotid stenosis and HHCY. OR: Odd ratio, CI: Confidence interval

Table 4: Generalized multifactor dimensionality reduction model of gene interaction

Model*	Training balance accuracy	Testing balance accuracy	Significant test (*P*)	CVC
8	0.5309	0.5033	4 (0.8281)	4/10
2,6	0.5539	0.4975	7 (0.1719)	6/10
2,6,12	0.5729	0.5175	7 (0.1719)	5/10
2,5,6,13	0.6001	0.4835	2 (0.9893)	2/10
3,4,6,8,14	0.6349	0.4708	3 (0.9453)	3/10
1,2,5,6,8,14	0.6752	0.4844	4 (0.8281)	2/10
1,2,4,5,7,8,14	0.7131	0.4935	4 (0.8281)	4/10
1,2,4,5,7,9,12,14	0.7498	0.5391	7 (0.1719)	5/10
2,3,4,5,6,7,9,12,14	0.7791	0.5288	8 (0.0547)	4/10
2,3,4,5,6,7,8,9,12,14	0.8009	0.5163	6 (0.3770)	2/10
1,2,3,4,5,6,7,8,12,13,14	0.8153	0.5216	5 (0.6230)	6/10
1,2,3,4,5,6,7,8,9,12,13,14	0.8273	0.5352	7 (0.1719)	8/10
1,2,3,4,5,6,7,8,9,11,12,13,14	0.8330	0.5347	6 (0.3770)	10/10
1,2,3,4,5,6,7,8,9,10,11,12,13,14	0.8353	0.5281	6 (0.3770)	10/10

*The numbers 1-14 represent rs10820385, rs10739871, rs10990616, rs10990618, rs79997970, rs4743634, rs7044631, rs7853468, rs10990647, rs78773706, rs10990654, rs16922693, rs384867, rs373637. CVC: Cross-validation consistency

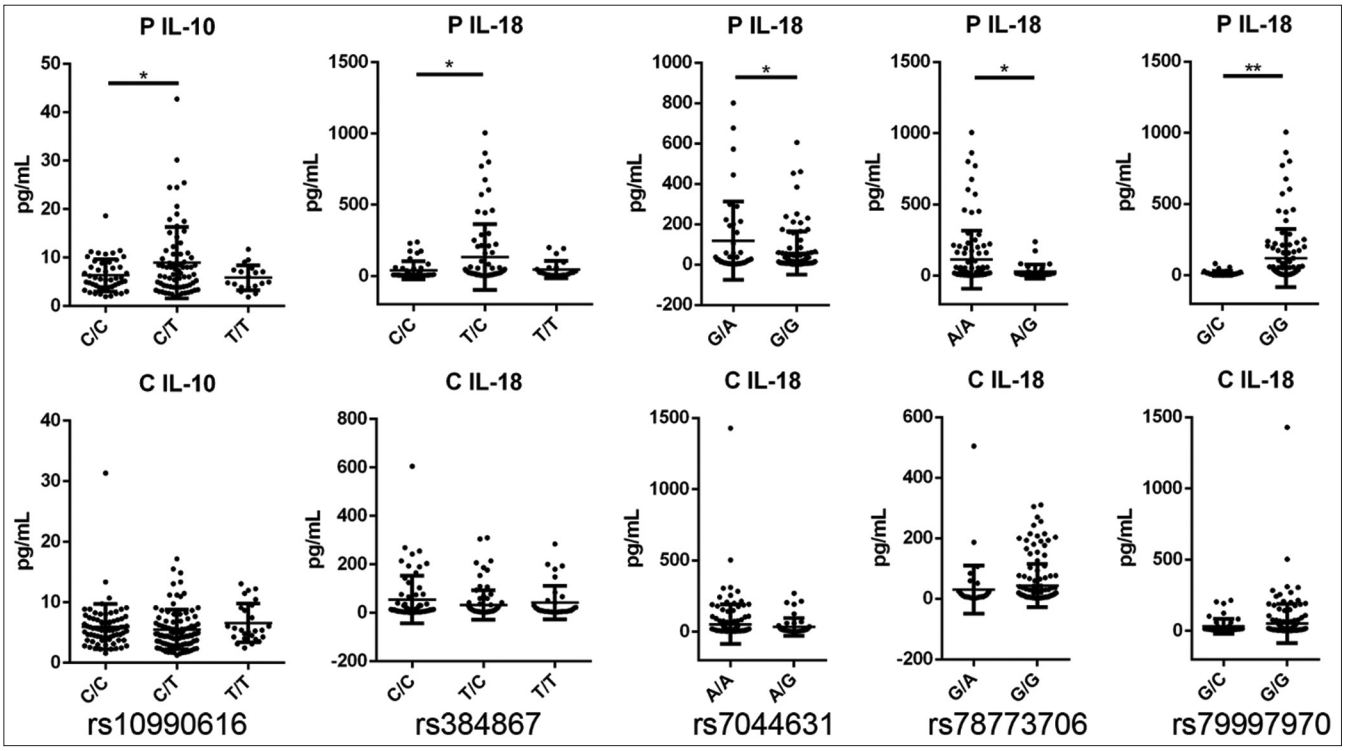

Figure 3: LINC01492 polymorphisms are associated with inflammation in LAA stroke patients. Values are shown as means ± standard deviation, **P* < 0.05, ***P* < 0.01. P: Patients, C: Controls, IL: Interleukin

gene transcription and the encoding of functional micropeptides.[24,25] Although this study has identified a correlation between the transcriptional level and genetic polymorphism of Linc01492 and LAA stroke, the underlying mechanism remains elusive. The aforementioned research provides valuable insights for our future investigations into mechanisms.

LINC01492 rs10820405 has been unequivocally associated with susceptibility to LAA stroke, wherein the G genotype confers an elevated risk compared to the A genotype (OR = 1.20, 95% CI = 1.12–1.28, $P = 4.51 \times 10^{-8}$).[4] The finding presented herein stems from data collected within the European population, a noteworthy aspect deserving emphasis. Considering the unique features of stroke in various geographical areas, exploring genetic variations and their association with the risk of LAA stroke in particular populations will contribute to the advancement of more accurate treatment approaches for LAA stroke.[26] The present study distinguishes itself from previous research by systematically investigating multiple genetic polymorphic loci of LINC01492, with the controls and patients sourced exclusively from The Third People's Hospital of Chengdu. The findings of this study hold significant implications for the prevention and treatment strategies targeting LAA stroke in the Chinese population.

Among the various inflammatory factors examined, only IL-10 and IL-18 exhibited significant associations with the genetic polymorphisms of LINC01492 investigated in this study. IL-10, primarily produced by Th2 cells, inhibits cytokine synthesis, particularly suppressing interferon-gamma (IFN-γ) production by Th1 cells. It exerts this effect by modulating antigen-presenting cells and reducing cytokine production in activated macrophages and dendritic cells.[10,27,28] IL-18, a pro-inflammatory cytokine from the IL-1 family, induces IFN-γ and regulates both Th1 and Th2 responses. It works with IL-12 to enhance Th1 responses and can stimulate Th2 cytokine production in the absence of IL-12. IL-18 is also involved in hemophagocytic lymphohistiocytosis, a cytokine-driven condition often triggered by infections.[29,30] Furthermore, the involvement of IL-10 and IL-18 in the pathogenesis of IS is highly relevant.[31] After considering the regulation of IL-10 and IL-18 by LINC01492 in the findings of this study, as well as the previously reported direct association between IL-10 and IL-18 with LAA stroke incidence, we intend to further investigate whether the impact of LINC01492 on susceptibility to LAA stroke is contingent on the regulation of IL-10 and IL-18.

However, this study has several limitations that may impact the interpretation of the results. First, being a single-center study, the population sample may not be broadly representative. Second, when examining the differences in inflammatory factors across various genotypes, we cannot exclude the possibility that other confounding factors may have influenced the results. Third, while significant differences in inflammatory factor levels were observed between genotypes, there is no direct evidence confirming that these variations are solely attributable to genetic differences. These limitations underscore the need for larger, multicenter studies and mechanistic research to strengthen the findings.

Author contributions

Jianyu Liu and Da Liu: Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Xing Guo: Conceived and designed the experiments. Li Zhou: Conceptualization. Zhiyao Xu: Performed the experiments; Analyzed and interpreted the data. Hua Liu: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ethical policy and institutional review board statement

This study obtained approval from the Ethics Committee of the Third People's Hospital of Chengdu in accordance with the Declaration of Helsinki (Approval No. 2019-S-110, dated on December 25th, 2019). All participants provided written informed consent before their participation.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: Primers for genotyping

SNPs	Primer types	Sequences
rs7853468	Specific primer G	TTACAGATGGTATAGAAAGGTAAATAACTCATCCACAG
	Specific primer A	TTTTACAGATGGTATAGAAAGGTAAATAACTCATCCACAA
	Common primer	CCAACATGTAGTAAGAAGTTTTAGTTTCTGGGTT
rs78773706	Specific primer A	TTGTAAAAACAGAATTTTAAGAATCTTCCAACAACCA
	Specific primer G	TTTTGTAAAAACAGAATTTTAAGAATCTTCCAACAATCG
	Common primer	AAGATTCTAACRTTATATGCATTGATTTGATTTTT
rs79997970	Specific primer G	CTCCTTTTTGTAATGACAAGCACAGATAAGG
	Specific primer C	TTCTCCTTTTTGTAATGACAAGCACAGATAAGC
	Common primer	TGAATTAAAAAAGGTTGTGGCAGGTT
rs10739871	Specific primer A	CTTTCTCGAACTTCAGGGGTATGTCTGCA
	Specific primer C	TTCTTTCTCGAACTTCAGGGGTATGTCTACC
	Common primer	AGCCAGGGTATGTGTTTTCCTGC
rs10739871	Specific primer A	CTTTCTCGAACTTCAGGGGTATGTCTGCA
	Specific primer C	TTCTTTCTCGAACTTCAGGGGTATGTCTACC
	Common primer	AGCCAGGGTATGTGTTTTCCTGC
rs10990616	Specific primer G	CCAAGKTATATGGCAAACTCAGGATCG
	Specific primer A	TTCCAAGKTATATGGCAAACTCAGGACCA
	Common primer	GAGAAACTGTTTTTGATATTAGATCAGAAAATACTGT
rs10990618	Specific primer G	TTCACCACCACAGAAGTCCCCTACTTCAGAG
	Specific primer A	TTTTCACCACCACAGAAGTCCCCTACTTCAGAA
	Common primer	ATAAGCAAAATAAAAGGAAACTAAAAGTGAAATTAGTT
rs10990647	Specific primer C	GAAAAACTCTTAAATTACAAACGTTGAGGGTACAAC
	Specific primer T	TTGAAAAACTCTTAAATTACAAACGTTGAGGGTACGAT
	Common primer	RCCGTCCCCTTTTCTATGATCAAGTT
rs10990654	Specific primer G	ACTTCATAGAATAACTTGGAGAGGTTTTATTTCCCTGTCTGTGTGTGTTCG
	Specific primer A	TTACTTCATAGAATAACTTGGAGAGGTTTTATTTCCCTGTCTGTGTGTGTTCA
	Common primer	TTTTCCTTTTTTTTTCTTTTTTTTGAGAGAAGGGGAAGTCTTTACAGTAT
rs16922693	Specific primer C	CCTTCCTAAATTAAGATTGAGCAATGATACAGATC
	Specific primer G	TTCCTTCCTAAATTAAGATTGAGCAATGATACAGATG
	Common primer	CCATCCTTTAGTAATGTTGTGACAGAGAATTT
rs373637	Specific primer G	GTCATAGATGGAATTTTGCTACCCTACG
	Specific primer A	TTGTCATAGATGGAATTTTGCTACCCTGCA
	Common primer	TAATACCACAATGACYTTCACCCCTT
rs384867	Specific primer T	TCTATTAAATGAGCTAAGGACTTGAATAGACATTTCTCTCAT
	Specific primer C	TCTATTTTAAATGAGCTAAGGACTTGAATAGACATTTCTCTCAC
	Common primer	GAACGCATACAAATGACCAACAGGAATTCGTCATTGTAGC
rs4743634	Specific primer T	CTGAACTAGTGTAAGGACTTAATGCCTCTACTTCGT
	Specific primer C	TTCTGAACTAGTGTAAGGACTTAATGCCTCTACTTTGC
	Common primer	ATCTGACACCCTACAATTCTTTCATCTTCTT
rs7044631	Specific primer G	CTTCAGAATTCATTCATTCACAGGTAAACTGTG
	Specific primer A	TTCTTCAGAATTCATTCATTCACAGGTAAACTGTA
	Common primer	TTGTCTAGAACATAGTACGATTTTTTGGTTTCTT

Supplementary Table 2: Primers for quantitative real-time-polymerase chain reaction

Gene name	Sequence
LINC01492	F: 5'-CCTTGCTTCTCAGGCTCC-3'
	R: 5'-AGGTAAAACCCTTGAAAATGTG-3'
β-actin	F: 5'-CCTTCCTGGGCATGGAGTC-3'
	R: 5'-TGATCTTCATTGTGCTGGGTG-3'

Supplementary Table 3: Hardy–Weinberg equilibrium of single nucleotide polymorphisms genotype in large artery atherosclerotic stroke and control group

SNPs	Genotype	LAA, *n* (%)	Control, *n* (%)	*P*
rs7853468	TT	63 (14.7)	78 (17.9)	0.969
	CC	231 (53.9)	209 (48.1)	
	TC	134 (31.3)	147 (33.8)	
rs78773706	AA	305 (71.2)	311 (71.6)	0.237
	AG	116 (27.1)	118 (27.1)	
	GG	7 (1.6)	5 (1.1)	
rs79997970	GG	318 (74.2)	324 (74.6)	0.768
	GC	105 (24.5)	104 (23.9)	
	CC	5 (1.1)	6 (1.3)	
rs10739871	CC	130 (30.3)	140 (32.2)	0.999
	AC	220 (51.4)	213 (49.0)	
	AA	78 (18.2)	81 (18.6)	
rs10820385	CC	322 (75.2)	332 (76.4)	0.951
	GC	100 (23.3)	96 (22.1)	
	GG	6 (1.4)	6 (1.3)	
rs10990616	TT	52 (12.1)	60 (13.8)	0.998
	TC	203 (47.4)	202 (46.5)	
	CC	173 (40.4)	172 (39.6)	
rs10990618	GG	330 (77.1)	330 (76.0)	0.946
	AG	86 (20.0)	96 (22.1)	
	AA	12 (2.8)	8 (1.8)	
rs10990647	GG	242 (56.5)	257 (59.2)	0.999
	AG	166 (38.7)	154 (35.4)	
	AA	20 (4.6)	23 (5.2)	
rs10990654	GG	360 (84.1)	390 (89.8)	0.756
	AG	59 (13.7)	42 (9.6)	
	AA	9 (2.1)	2 (0.4)	
rs16922693	GG	37 (8.6)	54 (12.4)	0.953
	GC	213 (49.7)	194 (44.7)	
	CC	178 (41.5)	186 (42.8)	
rs373637	TT	152 (35.5)	157 (36.1)	0.969
	TC	214 (50.0)	206 (47.4)	
	CC	62 (14.4)	71 (16.3)	
rs384867	CC	143 (33.4)	150 (34.5)	0.98
	TC	222 (51.8)	212 (48.8)	
	TT	63 (14.7)	72 (16.5)	
rs4743634	TT	296 (69.1)	290 (66.8)	0.99
	TC	119 (27.8)	129 (29.7)	
	CC	13 (3.0)	15 (3.4)	
rs7044631	GG	309 (72.1)	309 (71.1)	0.094
	AG	106 (24.7)	107 (24.6)	
	AA	13 (3.0)	18 (4.1)	

SNPs: Single nucleotide polymorphisms, LAA: Large artery atherosclerotic