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EDNRA affects susceptibility to large artery atherosclerosis stroke through potential inflammatory pathway

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This study aimed to explore the potential association between Endothelin type A receptor (EDNRA) genetic polymorphisms and susceptibility to large artery atherosclerotic stroke (LAA), as well as the involvement of inflammation mechanisms. We recruited Han Chinese patients with LAA and age- and sex-matched controls. The distribution of alleles and genotypes for 16 single nucleotide polymorphisms (SNPs) in EDNRA was analyzed using dominant, recessive, and co-dominant genetic models between cases and controls. We quantified the mRNA and protein levels of EDNRA and NLRP3 genes, and concentrations of inflammatory factors (TNFα, IL-1β, IL-6, IL-8, IL-10, IL-18, and CCL18) in peripheral blood samples randomly selected from cases and controls. We also investigated the relationship between these SNPs, gene expression patterns and inflammatory factor levels. A total of 428 LAA cases and 434 controls were enrolled in this study. The results showed that rs5343 TT genotype of EDNRA was significantly associated with an increased risk of LAA (OR = 3.243, 95%CI = 1.608–6.542, $P = 0.001$). It also demonstrated a significant upregulation level of NLRP3 as well as higher concentrations of IL-10, IL-18, and CCL-18 in cases compared to controls. Besides, we discovered that the EDNRA polymorphisms were linked to NLRP3, IL-6, IL-10, and IL-18 levels in cases. There existed a positive correlation between EDNRA transcription levels and both NLRP3 transcript levels ($r = 0.437$, $p < 0.001$) and IL-18 concentrations ($r = 0.212$, $p < 0.001$). EDNRA is linked to susceptibility of LAA. This association may be attributed to the NLRP3-mediated inflammatory pathway.

Stroke ranks as the second leading cause of death and the third leading cause of death and disability combined worldwide[1,2]. In China, stroke remains a severe burden and is still the primary cause of mortality[3]. Furthermore, without appropriate prevention strategies in place, the incidence of stroke is likely to continue its upward trend. Ischemic stroke (IS) constituted 62.4% of all incident strokes[1]. Among the subtypes of IS, large artery atherosclerotic stroke (LAA) is the most prevalent and its incidence is increasing at a rate of 5.7% annually[4].

In addition to providing effective treatment for patients with IS to minimize mortality and morbidity, prevention of IS occurrence is a practical and efficacious approach towards reducing the burden of stroke[5]. The reduction in IS incidence through targeted interventions aimed at single or multiple risk factors at both population and individual levels constitutes IS prevention, which necessitates the identification of risk factors for effective implementation[6].

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Traditional IS risk factors comprise age, gender, hypertension, diabetes mellitus, heart diseases, hyperlipidemia, smoking and drinking habits, high body mass index (BMI), elevated homocysteine levels and carotid stenosis[7–9]. Additionally, significant traditional risk factors include high fasting blood glucose levels and exposure to environmental particulate matter pollution[1]. Hypertension and hyperlipidemia are prevalent risk factors for IS, both of which are closely associated with atherosclerosis[10]. In addition to the conventional factors, there exists a hereditary component in IS etiology. Unraveling the genetic contributions to IS could facilitate a more precise definition of causal pathways, identification of novel therapeutic targets, and improved options for diagnosis and prognosis[10–12]. The identification of genetic risk factors for LAA is particularly valuable, given the higher estimated heritability of this type of stroke compared to other subtypes[4].

Endothelin type A receptor (EDNRA) is a receptor for the potent vasoconstrictor endothelin-1 (ET-1), which is primarily expressed in vascular smooth muscle cells[13]. It is now widely used in clinical practice as a therapeutic target for drugs for cardiovascular diseases such as pulmonary hypertension and hypertension[14,15]. EDNRA promotes the proliferation and migration of vascular smooth muscle cells, inducing the formation of extracellular matrix and fibers that are involved in the development and progression of atherosclerosis[16,17]. Studies on the EDNRA SNP rs1878406 have demonstrated that polymorphism at this locus is associated with carotid plaque and an increased risk of coronary artery disease[18,19]. Furthermore, endothelin (EDNs) and EDNRA are implicated in the inflammatory process which also plays a role in LAA pathogenesis[20–22]. Therefore, we propose that EDNRA may be involved in the pathogenesis of LAA. Consistent with our expectations, a recent study has demonstrated that the rs1878406 polymorphism of the EDNRA gene is significantly associated with susceptibility to LAA[23]. However, to the best of our knowledge, no systematic studies have comprehensively analyzed genetic polymorphisms of EDNRA and their potential association with LAA risk. In the present study, we selected and genotyped 16 tagSNPs in the EDNRA gene using SNPscan technology to examine the correlation between genetic polymorphisms of EDNRA and LAA risk. Additionally, we investigated whether these relevant genetic polymorphisms regulate inflammatory pathways that influence LAA pathogenesis.

Materials and methods

Ethics statement

This study was approved by the Ethics Committee of the Third People's Hospital of Chengdu in accordance with the Declaration of Helsinki (2019-S-110). Prior to participation, all participants provided written informed consent. This trial was registered at the Chinese Clinical Trial Registry (www.chictr.org.cn) (trial registration number ChiCTR2000032684).

Study populations

We consecutively recruited first-ever LAA patients from the neurology department of the Third People's Hospital of Chengdu. LAA was diagnosed using Chinese ischemic stroke subclassification[24]. Control subjects matched by age, gender, and ethnic group were randomly selected from the healthy adults who underwent periodical medical check-ups at the Physical Examination Center of the same Hospital during the same period when patients were recruited. The controls were healthy, without any vascular or neurological diseases by questionnaires, history-taking, and clinical examination. The demographic data and risk factors were recorded in detail, including age, gender, hypertension, diabetes, heart diseases, hyperlipidemia, smoking status, drinking habits, overweight, carotid stenosis, and high homocysteine (HHCY) levels. All data were registered and kept confidential by two staff in the department.

Variants definition

The demographic characteristics, potential stroke risk factors, and medical history were gathered from each participant for analysis. Cigarette smoking was defined as the habitual consumption of at least one cigarette per day for a minimum duration of one year[25]. The presence of alcohol consumption was defined as drinking alcohol at least 12 times during the past year[25]. The diagnosis of hypertension was established based on the guidelines provided by the World Health Organization (WHO) and the International Society of Hypertension, which define hypertension as blood pressure equal to or exceeding 140/90 mmHg, or through the administration of antihypertensive medications[26,27]. Diabetes mellitus was diagnosed based on elevated fasting plasma glucose levels (≥ 7.0 mM), or a documented history of the oral hypoglycemic agent or insulin treatment, in accordance with the criteria set by the WHO[28]. Dyslipidemia was defined according to the 2016 Chinese guideline for the management of dyslipidemia in adults[29]. The condition of being overweight is defined as having a body mass index equal to or greater than 24 kg/m^2 according to the Chinese criteria[30]. The definition of HHCY is a plasma homocysteine concentration greater than 10 umol/L[31]. The definition of carotid stenosis is the presence of more than 50% blockage as determined by angiography[32].

SNPs selection and genotyping

Genotypes for SNPs in EDNRA representing the Han Chinese were obtained from the HapMap database (phase II, 8 November, on NCBI B36 assembly, dbSNP b126). We carefully selected 16 tagSNPs (rs1878406, rs6842241, rs6841581, rs1801708, rs10305863, rs702757, rs7657903, rs10305895, rs908581, rs7655892, rs78047355, rs2048894, rs5333, rs5335, rs5342 and rs5343) based on their high linkage disequilibrium with r$^2 > 0.8$ and a minor allele frequency > 0.05 in the Chinese Han population.

A total of 5mL venous blood was collected from the antecubital vein in the morning after an overnight fast using EDTA (Disodium salt, 50 mmol/L) tubes. The collected samples were subsequently frozen at -80 °C until testing was conducted. Genomic DNA was extracted from peripheral blood using the Genomic DNA Extraction Kit (Tiangen)[33]. The genotypes of selected SNPs were determined using SNPscan technology (Genesky)[34]. Briefly, the high specificity of the ligase ligation reaction is utilized for SNP loci allele identification, followed by

introducing non-specific sequences at the end of the ligation probe and performing a ligase addition reaction to obtain ligated products with varying lengths. The ligated products were amplified by PCR using fluorescence-labeled universal primers, and the resulting amplicons were separated via fluorescence capillary electrophoresis (Table 1). Finally, the genotypes of each SNP locus were determined through analysis of the electrophoretic profiles.

SNPs	Primer types	Sequences
rs1878406	Specific primer C	ACAAATTAAAATGCATAATCAAAATCAAGCCTGTTAGAGAC
	Specific primer T	ACAGGAATTTTAAAATGCATAATCAAAATCAAGCCTGTTAGAGAT
	Common primer	AAAATTCATCCATTAATAGCAACTAAATTTCAAAATTGAAGCTGT
rs6842241	Specific primer C	TTTTCTGGTTGGCTGATTCTCCCACC
	Specific primer A	TTTTTTCTGGTTGGCTGATTCTCCCACA
	Common primer	TAGAGAAGCTGTAAGATTAGTGAACAGGGTT
rs6841581	Specific primer G	ACAAGTCTTTGTGGAGAGACGCTCG
	Specific primer A	TTACAAGTCTTTGTGGAGAGACGCTCA
	Common primer	TGGAGAAAAACTAACACTCAACACCAT
rs1801708	Specific primer G	TTTTTGGGAGAAGCAGTGCCCAGTAG
	Specific primer A	TTTTTTTGGGAGAAGCAGTGCCCAGCAA
	Common primer	GTTTTCTGAAGCCGGGGAAGTTTTTT
rs10305863	Specific primer G	TGTAATTCAGGTTCTTATCAGCCTGAATGTACAG
	Specific primer C	TTTGTAATTCAGGTTCTTATCAGCCTGAATGTACAC
	Common primer	AAAGAAGTAACTACTACTTAAAGGAAAAACTGACRTAG
rs702757	Specific primer A	CAGCAGGGAGTTATAAATGCAGAGATCGA
	Specific primer T	TTCAGCAGGGAGTTATAAATGCAGAGATCGT
	Common primer	TAATTATTTTCTTGAGCCATGATGGGTT
rs7657903	Specific primer C	ATATTTCCCTTCTCTCCCCTTCCCATTACC
	Specific primer T	TTTATATTTCCCTTCTCTCCCCTTCCCATTGCT
	Common primer	AACCCCTGGCAACCATTAATCTGTTAT
rs10305895	Specific primer A	GTCCTGGGCATCACAATCTTTATAAGCA
	Specific primer G	TTGTCCTGGGCATCACAATCTTTATAAACG
	Common primer	TCTCAGTCTGTGCTGTTGGAAGATGT
rs908581	Specific primer G	TTAATAAAAAGAAAAATCATATAGGAAGAACATTTG
	Specific primer A	TTTTAATAAAAAGAAAAATCATATAGGAAGAACATCTA
	Common primer	TGTAGGACACATTGTTCTTTTTTCAAAATTT
rs7655892	Specific primer C	TTTATGAGCTGAATAAATTATGGCCCAGTAC
	Specific primer T	TTTTTATGAGCTGAATAAATTATGGCCCAGCAT
	Common primer	AAAGAGTTCCCATGATTTTTTTACACAGTTT
rs78047355	Specific primer A	GTAAAGCATTTGCAGCTGTCTTTTAATGCA
	Specific primer G	TTGTAAAGCATTTGCAGCTGTCTTTTAATACG
	Common primer	CCACACCTGATTCTCTTCTAAATTGGT
rs2048894	Specific primer G	TCATCTCTAGGGAATTCTGGTGAAGAAAGAG
	Specific primer A	TTTCATCTCTAGGGAATTCTGGTGAAGAAAGAA
	Common primer	TCAACATAATTCTAAGAGTTGTGTTTTCACATT
rs5333	Specific primer T	TGCTCTTTGCTGGTTCCCTCTTGAT
	Specific primer C	TTTGCTCTTTGCTGGTTCCCTCTTGAC
	Common primer	TTAAGCCGTATATTGAAGAAAACTGTGTATAACGTTT
rs5335	Specific primer G	GGAGAAAAAAATCACAAGGCAACTGTCAG
	Specific primer C	TTGGAGAAAAAAATCACAAGGCAACTGTCAC
	Common primer	TCCGGGAATCTCTTCTCTGATCC
rs5342	Specific primer T	TATGTCACTATTACAAATACCTGAATGAAAATTGCT
	Specific primer C	TTTATGTCACTATTACAAATACCTGAATGAAAATTGCC
	Common primer	GAATCATCTGAAAAATTCGATCTTTTTCTTT
rs5343	Specific primer G	GGGAGTTAGGTTCATACTGAAAACCCCTAG
	Specific primer A	TTGGGAGTTAGGTTCATACTGAAAACCCCCAA
	Common primer	AAGTGAAGAATCGTTAAGTTCTGGTGGTT

Table 1. Primers for genotyping.

Quantitative real-time PCR

We randomly selected a subset of LAA patients and control samples to assess the transcript levels of EDNRA and NLRP3. Total RNA was isolated from venous blood by using the TRNzol Universal Total RNA Isolation Kit (Tiangen)[35]. The quality of total RNA was tested by agarose gel electrophoresis. 1 μg of total RNA was reverse transcribed by the use of TransScript® Uni All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (Transgen). The resultant cDNA was diluted 10-fold before PCR amplification. A reverse transcriptase minus reaction served as a negative control. The mRNA levels were measured by SYBR green real-time PCR (Yeasen)[36]. Primer sequences of genes used for qRT-PCR analysis are given in Table 2.

Inflammation-related factors assay

Plasma was collected using EDTA as an anticoagulant, and samples were centrifuged at 1000×g for 15 min at a temperature of 4 °C. The levels of TNF-α, CCL18, IL-1β, IL-6, IL-8, IL-10, and CIL-18 were quantified by flow cytometry in accordance with the manufacturer's instructions (Nuohebio).

Enzyme-linked immunosorbent assay (ELISA)

Plasma was collected using EDTA as an anticoagulant, and samples were centrifuged at 1000×g for 15 min at a temperature of 4 °C. Store the sample at -80 °C to prevent repeated freezing and thawing. The contents of NLRP3 were determined strictly following the instructions of the manufacturers (ABclonal).

Statistical analysis

All statistical tests were analyzed using SPSS statistics version 21.0. The chi-square test was utilized to assess the proportions of clinical and environmental variables, as well as Hardy-Weinberg equilibrium (HWE)[37]. Subsequent genetic association analyses were conducted using three genetic models: dominant, recessive, and allelic comparison. Normally distributed, continuous data were compared with a student's t-test and expressed as mean ± standard deviation. The BH (Benjamini–Hochberg) method of FDR (False discovery Rate) was used to correct type I errors[38]. The generalized multifactor dimensionality reduction (GMDR) beta v0.9 software package was used to analyze gene–gene interactions[39]. GMDR software obtains the best model combination from multiple genes and behavioral indicators through the factor dimensionality reduction principle. The optimal model is obtained from the following results: (1) The model is meaningful only when the p-value is less than 0.05; (2) The larger the testing balance accuracy is, the better the model effect is; (3) The closer the cross validation (CV) consistency is to 10, the better. The influence of high-risk interactive genotypes on functional outcomes was investigated with multivariable logistic regression analysis, after adjusting for the main baseline variables related to each main variable in the univariate analysis (enter approach and probability of entry $p < 0.2$)[40]. A p-value of less than 0.05 was considered a statistically significant difference.

Results

Baseline characteristics of the subjects

In this study, a total of 434 patients with LAA and 428 controls were enrolled. The baseline clinical and demographic characteristics are presented in Table 3. Compared to the control group, the patients were significantly older ($p < 0.001$) and had a higher proportion of males ($p = 0.027$). Multi-factor logistic regression analysis revealed significant differences in traditional risk factors for IS, such as gender ($p = 0.027$), hypertension ($p < 0.001$), diabetes ($p < 0.001$), heart diseases ($p = 0.006$), smoking status ($p < 0.001$), and carotid stenosis ($p = 0.021$).

Genotypic and allelic frequencies

The observed frequency distribution of the sixteen variants conforms to Hardy-Weinberg equilibrium (HWE) ($p > 0.05$), indicating that the gene frequencies in the selected study population are representative of those in the general population (Table 4).

EDNRA rs5343 SNP and the risk of LAA

From univariate analysis, three SNP candidates (rs5343, rs2048894, and rs908581) were selected for comparison with logistic regression analysis between the LAA cases and control groups based on gender, hypertension, diabetes, heart diseases, smoking status, drinking habits, overweightness, carotid stenosis and HHCY. The CC genotype of rs5343 was associated with a lower risk of LAA (OR = 0.734, 95%CI = 0.539–0.999, P = 0.049) while the TT genotype of rs5343 was linked to an increased risk (OR = 3.243, 95%CI = 1.608–6.542, P = 0.001) (Table 5). We also analyzed the association between rs5343 gene polymorphisms and cardiovascular risk factors such as hypertension, diabetes, heart diseases, and carotid stenosis, however, no statistically significant correlation was found (Supplementary Table 1).

Gene name	Sequence	
NLRP3	F:5'-TGCCATCATGAAGCCAGAGA-3'	R:5'-AACATCGCAGTCTCCAAGGA-3'
EDNRA	F:5'-ATCCCGGACTTCAGATCCCC-3'	R:5'-CAACATGAAAAAGGGCTTGGG-3'
β-actin	F:5'-TCACACGAGATGAGCTTAGGGCAA-3'	R:5'-TACAGTTCTGGGCGGCGACTTTAT-3'

Table 2. Primers for qRT-PCR.

Characteristics	LAA($n=428$)	Controls($n=434$)	Unadjusted		Adjusted*	
			OR (95% CI)	p	OR (95% CI)	p
Age, years	63.33 ± 10.54	58.45 ± 11.71		< 0.001		
Male/female	274/154	189/245	2.306(1.754–3.033)	< 0.001	1.503(1.048–2.157)	0.027
Hypertension, n (%)	321(75.0)	175(40.3)	4.440(3.319–5.939)	< 0.001	2.984(2.158–4.128)	< 0.001
Diabetes, n (%)	180(42.1)	67(15.4)	3.976(2.877–5.494)	< 0.001	2.694(1.886–3.849)	< 0.001
Heart diseases, n (%)	47(11.0)	18(4.1)	2.851(1.627–4.995)	< 0.001	2.421(1.295–4.525)	0.006
Hyperlipidemia, n (%)	288(67.3)	276(63.6)	1.178(0.889–1.560)	0.254	1.041(0.753–1.440)	0.806
Smoking, n (%)	160(37.4)	65(15.0)	3.389(2.440–4.707)	< 0.001	2.329(1.427–3.800)	< 0.001
Drinking, n (%)	129(30.1)	56(12.9)	2.912(2.055–4.126)	< 0.001	1.078(0.650–1.786)	0.771
Overweight, n (%)	238(55.6)	186(42.9)	1.670(1.276–2.186)	< 0.001	1.318(0.966–1.799)	0.082
HHCY, n (%)	282(65.9)	227(52.3)	1.761(1.338–2.318)	< 0.001	1.034(0.743–1.439)	0.843
Carotid stenosis, n (%)	257(60.0)	165(38.0)	2.450(1.863–3.222)	< 0.001	1.450(1.057–1.989)	0.021

Table 3. Baseline characteristics of the study population. Notes: *Adjusted for gender, hypertension, diabetes, heart diseases, smoking status, drinking habits, overweightness, carotid stenosis and HHCY.

Linkage disequilibrium (LD) analysis and haplotype block structure

As illustrated in Fig. 1, the LD analysis identified four blocks among the 16 tagSNPs. To minimize potential false positives, haplotypes with frequencies less than 0.01 were excluded from the haplotype analysis. After adjusting for gender, hypertension, diabetes, heart diseases, smoking status, drinking habits, overweightness, carotid stenosis and HHCY, no significant association was observed between any of the haplotypes and LAA risk (Table 6).

SNP-SNP interactions

The GMDR model identified the gene-gene interaction between rs2048894 and rs5343 as the most optimal, with a cross-validation consistency of 10/10 and a sign test result of 9 ($p=0.0107$) (Table 7). The optimal interaction model between rs2048894 and rs5343 by GMDR is illustrated in Fig. 2. After adjusting for hypertension, diabetes, heart disease, hyperlipidemia, smoking status, drinking habits, overweight, carotid stenosis and HHCY through multivariate logistic regression analysis, it was determined that the combination of rs2048894 and rs5343 is significantly associated with an increased risk of IS (OR = 2.028, 95%CI = 1.189–3.457, $p=0.0101$).

Inflammatory pathways and LAA pathogenesis

Numerous reports have confirmed the impact of cardiovascular diseases, including LAA, on inflammatory pathways and factor levels[41–44]. Consistent with prior research, this study's findings also indicate significant distinctions in NLRP3(Ctr = 282.9 ± 58.75, LAA = 541.4 ± 92.75, pg/mL, $p < 0.05$), CCL18 (Ctr = 4015 ± 20.37, LAA = 4126 ± 19.24, pg/mL, $p < 0.0001$), IL-10 (Ctr = 5.74 ± 0.21, LAA = 6.56 ± 0.28, pg/mL, $p < 0.05$), and IL-18 (Ctr = 15.54 ± 1.39, LAA = 20.60 ± 1.62, pg/mL, $p < 0.05$) between the LAA and control groups (Fig. 3).

EDNRA polymorphism is associated with inflammation

We investigated the correlation between genetic variation and inflammation at multiple SNP loci in EDNRA. Some genotypes were excluded from the analysis due to low population frequency. In LAA patients, NLRP3 expression levels were significantly higher in those with the rs5342 GG genotype compared to the AA and AG genotypes. Patients with the rs6841581 GG genotype exhibited higher IL-6 levels than those with the GA genotype The rs6842241 locus, in complete linkage disequilibrium with rs6841581, showed higher IL-6 levels in patients with the CC genotype compared to the CA genotype. Patients carrying the rs7657903 GA genotype had elevated IL-8 levels compared to those with the GG genotype. Carriers of the rs702757 TT genotype had significantly higher IL-10 levels compared to TA genotype carriers, with no significant difference between AA genotype carriers and others at this locus. Conversely, patients with the rs5343 CT genotype had higher IL-18 concentrations than those with the CC genotype ($p < 0.05$). (Fig. 4).

The expression of EDNRA is positively associated with the expression of NLRP3 and the levels of IL-18 in patients

To further investigate the association between EDNRA and inflammatory pathways, we conducted an analysis of the correlation between EDNRA expression levels and NLRP3, CCL18, as well as various inflammatory factors. We observed a significant correlation between the expression levels of EDNRA and NLRP3 in both LAA patients (Pearson's $r=0.437$, $p < 0.001$) and controls (Pearson's $r=0.234$, $p=0.002$). Notably, the correlation between EDNRA and NLRP3 was more pronounced in LAA patients compared to controls. Furthermore, only LAA patients showed a correlation between EDNRA and IL-18 levels (Pearson's $r=0.212$, $p=0.009$), while no significant correlations were found between EDNRA expression levels and CCL18, IL-6, IL-8 or IL-10 levels in either patient group or controls (Fig. 5) (Table 8).

SNPs	Genotype	LAA(n%)	p	Controls(n%)	p
rs10305863	G/G	358(83.6)	0.886	358(82.5)	0.942
	C/C	4(0.9)		3(0.7)	
	G/C	66(15.4)		73(16.8)	
rs10305895	A/A	209(48.8)	0.824	228(52.5)	0.98
	G/G	35(8.2)		34(7.8)	
	A/G	184(43.0)		172(39.6)	
rs5333	T/T	261(61.0)	0.454	288(66.4)	0.867
	C/C	15(3.5)		17(3.9)	
	T/C	152(35.5)		129(29.7)	
rs5335	G/G	141(32.9)	0.932	133(30.6)	0.665
	C/C	81(18.9)		95(21.9)	
	G/C	206(48.1)		206(47.5)	
rs5342	A/A	159(37.1)	0.504	160(36.9)	0.934
	G/G	56(13.1)		70(16.1)	
	A/G	213(49.8)		204(47.0)	
rs5343	C/C	212(49.5)	0.842	247(56.9)	0.073
	T/T	34(7.9)		15(3.5)	
	C/T	182(42.5)		172(39.6)	
rs1801708	G/G	185(43.2)	0.671	193(44.5)	0.831
	A/A	44(10.3)		44(10.1)	
	G/A	199(46.5)		197(45.4)	
rs1878406	C/C	240(56.1)	0.617	253(58.3)	0.966
	T/T	22(5.1)		23(5.3)	
	C/T	166(38.8)		158(36.4)	
rs2048894	G/G	261(61.0)	0.33	299(68.9)	0.471
	A/A	14(3.3)		17(3.9)	
	G/A	153(35.7)		118(27.2)	
rs6841581	G/G	240(56.1)	0.497	252(58.1)	0.949
	A/A	21(4.9)		23(5.3)	
	G/A	167(39.0)		159(36.6)	
rs6842241	C/C	240(56.1)	0.497	252(58.1)	0.949
	A/A	21(4.9)		23(5.3)	
	C/A	167(39.0)		159(36.6)	
rs702757	T/T	186(43.5)	0.868	195(44.9)	0.827
	A/A	46(10.7)		43(9.9)	
	T/A	196(45.8)		196(45.2)	
rs7655892	C/C	330(77.1)	0.389	347(80.0)	0.637
	T/T	3(0.7)		7(1.6)	
	C/T	95(22.2)		80(18.4)	
rs7657903	G/G	362(84.6)	0.898	358(82.5)	0.786
	A/A	2(0.5)		5(1.2)	
	G/A	64(15.0)		71(16.4)	
rs78047355	A/A	393(91.8)	0.958	399(91.9)	0.681
	G/G	1(0.2)		0(0.0)	
	A/G	34(7.9)		35(8.1)	
rs908581	C/C	258(60.3)	0.871	293(67.5)	0.949
	T/T	19(4.4)		17(3.9)	
	C/T	151(35.3)		124(28.6)	

Table 4. Hardy-Weinberg equilibrium of SNPs genotype in LAA and control group.

Discussion

In this study, the pathogenesis of LAA was found to be associated with traditional risk factors including age, gender, hypertension, diabetes, heart disease, smoking and carotid artery stenosis. The EDNRA rs5343 gene polymorphism was found to be significantly associated with the risk of LAA development under all genetic models, however, this SNP was not associated with vascular risk factors for LAA (including hypertension, diabetes, heart diseases, and carotid stenosis). These results suggested that the rs5343 polymorphism might

SNP	Genetic models		Unadjusted OR (95% CI)	p	Adjusted* OR (95% CI)	p
rs10305863	Dominant	GG/(CC+GC)	1.086(0.760–1.550)	0.651	1.028(0.681–1.551)	0.895
	Recessive	CC/(GG+GC)	1.355(0.302–6.092)	0.691	1.462(0.268–7.980)	0.661
	Codominant	C/G	0.945(0.678–1.317)	0.739		
rs10305895	Dominant	AA/(GG+AG)	0.862(0.660–1.126)	0.277	0.934(0.688–1.267)	0.660
	Recessive	GG/(AA+AG)	1.048(0.641–1.714)	0.853	0.948(0.540–1.663)	0.852
	Codominant	G/A	1.104(0.896–1.360)	0.353		
rs5333	Dominant	TT/(CC+CT)	0.792(0.600–1.046)	0.101	0.839(0.611–1.153)	0.279
	Recessive	CC/(TT+CT)	0.891(0.439–1.808)	0.749	0.827(0.371–1.843)	0.642
	Codominant	C/T	1.168(0.922–1.479)	0.198		
rs5335	Dominant	GG/(CC+GC)	1.112(0.835–1.481)	0.469	1.184(0.853–1.645)	0.313
	Recessive	CC/(GG+GC)	0.833(0.598–1.161)	0.280	0.781(0.533–1.143)	0.203
	Codominant	C/G	0.899(0.743–1.087)	0.271		
rs5342	Dominant	AA/(GG+AG)	1.012(0.768–1.335)	0.931	1.134(0.826–1.557)	0.437
	Recessive	GG/(AA+AG)	0.783(0.535–1.145)	0.205	0.722(0.464–1.124)	0.150
	Codominant	G/A	0.932(0.768–1.132)	0.478		
rs5343	Dominant	CC/(TT+CT)	0.743(0.568–0.972)	0.030	0.734(0.539–0.999)	0.049
	Recessive	TT/(CC+CT)	2.410(1.293–4.494)	0.004	3.243(1.608–6.542)	0.001
	Codominant	T/C	1.360(1.096–1.687)	0.005		
rs1801708	Dominant	GG/(AA+AG)	0.951(0.726–1.244)	0.712	1.012(0.744–1.377)	0.938
	Recessive	AA/(GG+AG)	1.016(0.653–1.579)	0.945	0.992(0.604–1.630)	0.976
	Codominant	A/G	1.032(0.844–1.261)	0.760		
rs1878406	Dominant	CC/(TT+CT)	0.913(0.697–1.196)	0.510	0.988(0.724–1.348)	0.940
	Recessive	TT/(CC+CT)	0.968(0.531–1.765)	0.916	0.863(0.447–1.664)	0.659
	Codominant	T/C	1.058(0.848–1.320)	0.617		
rs2048894	Dominant	GG/(AA+AG)	0.706(0.533–0.935)	0.015	0.731(0.531–1.007)	0.055
	Recessive	AA/(GG+AG)	0.829(0.404–1.705)	0.611	0.746(0.329–1.688)	0.481
	Codominant	A/G	1.263(0.994–1.606)	0.056		
rs6841581	Dominant	GG/(AA+AG)	0.922(0.704–1.208)	0.555	0.988(0.724–1.348)	0.939
	Recessive	AA/(GG+AG)	0.922(0.502–1.692)	0.793	0.809(0.419–1.564)	0.529
	Codominant	A/G	1.045(0.838–1.303)	0.698		
rs6842241	Dominant	CC/(AA+CA)	0.922(0.704–1.208)	0.555	0.988(0.724–1.348)	0.939
	Recessive	AA/(CC+CA)	0.922(0.502–1.692)	0.793	0.809(0.419–1.564)	0.529
	Codominant	A/C	1.045(0.838–1.303)	0.698		
rs702757	Dominant	TT/(AA+AT)	0.942(0.720–1.233)	0.663	1.016(0.747–1.382)	0.920
	Recessive	AA/(TT+AT)	1.095(0.706–1.698)	0.685	1.092(0.668–1.783)	0.726
	Codominant	A/T	1.054(0.862–1.288)	0.610		
rs7655892	Dominant	CC/(TT+CT)	0.844(0.610–1.169)	0.308	0.889(0.612–1.291)	0.537
	Recessive	TT/(CC+CT)	0.431(0.111–1.676)	0.211	0.324(0.077–1.360)	0.124
	Codominant	T/C	1.102(0.817–1.484)	0.525		
rs7657903	Dominant	GG/(AA+AG)	1.164(0.812–1.670)	0.408	1.108(0.729–1.683)	0.630
	Recessive	AA/(GG+AG)	0.403(0.078–2.088)	0.263	0.496(0.073–3.352)	0.472
	Codominant	A/G	0.838(0.598–1.175)	0.305		

Continued

SNP	Genetic models		Unadjusted		Adjusted*	
			OR (95% CI)	p	OR (95% CI)	p
rs78047355	Dominant	AA/(GG+AG)	0.985(0.604–1.606)	0.952	0.921(0.526–1.614)	0.775
	Recessive	GG/(AA+AG)	0.998(0.993–1.002)	0.236	/	/
	Codominant	G/A	1.045(0.650–1.680)	0.856		
rs908581	Dominant	CC/(TT+CT)	0.730(0.553–0.965)	0.027	0.831(0.604–1.143)	0.256
	Recessive	TT/(CC+CT)	1.140(0.584–2.223)	0.702	0.912(0.438–1.901)	0.806
	Codominant	T/C	1.273(1.005–1.613)	0.045		

Table 5. Association of the EDNRA polymorphism with risk of LAA. Notes: To analyze the associations between SNPs and LAA risk, we utilized dominant, recessive, and codominant models. The EDNRA SNPs possess two alleles: A (major allele) and a (minor allele). These three models can be defined as follows: the dominant model (AA versus aa+aA), the recessive model (aa versus aa+aA), and the codominant model (a allele versus A allele). * Adjusted for gender, hypertension, diabetes, heart diseases, smoking status, drinking habits, overweightness, carotid stenosis and HHCY.
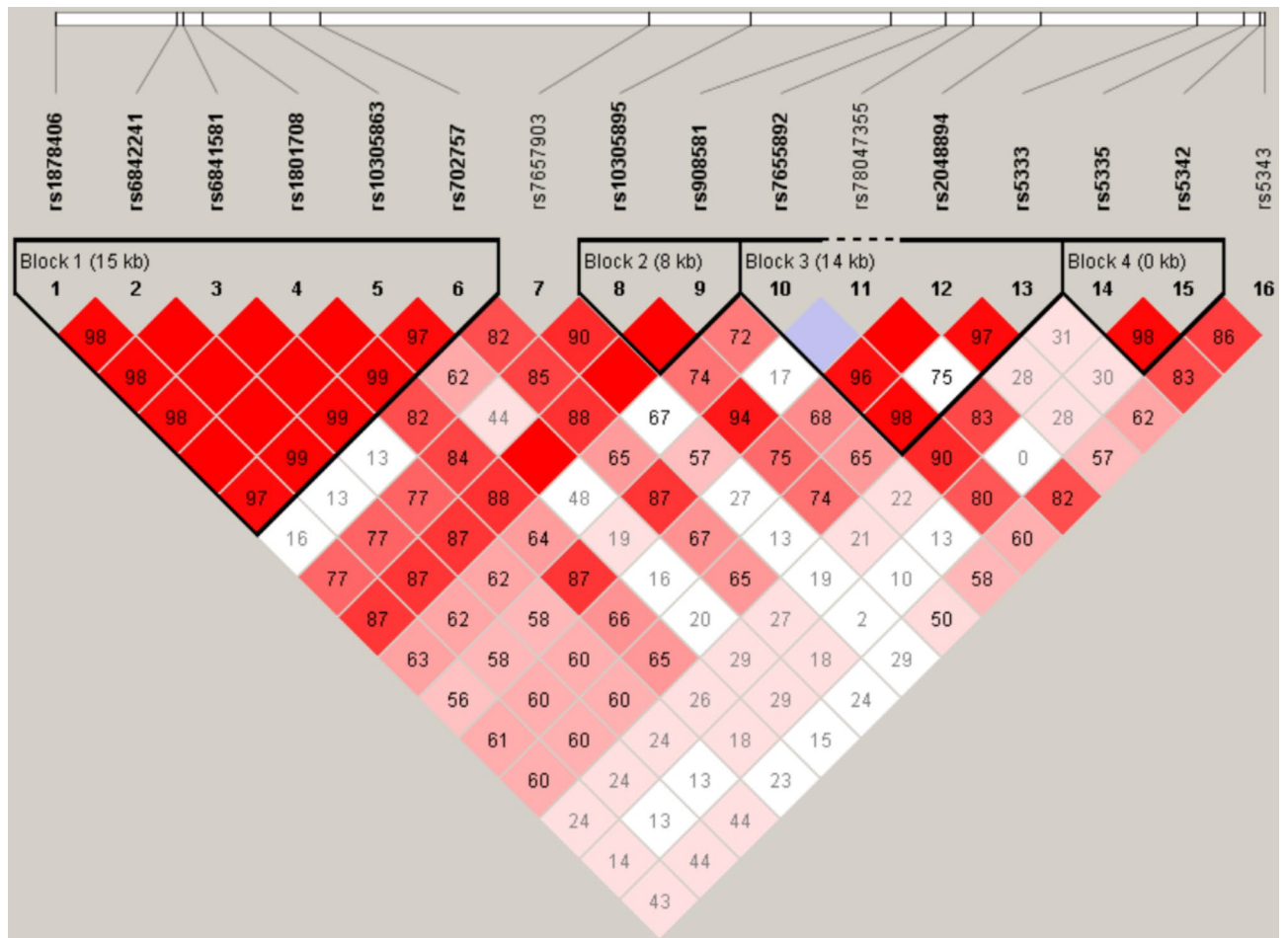
Fig. 1. LD and correlation coefficients (r^2) among sixteen selected SNPs of the EDNRA gene.

HapMap block	Haplotype[*]	Cases Freq	Controls Freq	Adjusted OR (95% CI)	P[**]
Block 1	CCGGGT	0.6577	0.6705	1.00	/
	TAAAGA	0.24	0.2327	1.03 (0.79–1.33)	0.85
	CCGACA	0.0859	0.0887	0.96 (0.65–1.42)	0.84
Block 2	AC	0.7033	0.7235	1.00	/
	GT	0.2208	0.182	0.91 (0.69–1.19)	0.49
	GC	0.0759	0.0945	1.12 (0.76–1.67)	0.56
Block 3	CGT	0.7815	0.811	1.00	/
	TAC	0.1156	0.1021	0.90 (0.64–1.28)	0.56
	CAC	0.0899	0.0718	0.78 (0.52–1.15)	0.21
Block 4	GA	0.5664	0.5401	1.00	/
	CG	0.376	0.3926	1.18 (0.94–1.48)	0.16
	CA	0.0539	0.0636	1.13 (0.72–1.78)	0.59

Table 6. Association between haplotype and the risk of LAA. Notes: [*]Haplotypes with frequency < 1% were omitted, [**]Adjusted for gender, hypertension, diabetes, heart diseases, smoking status, drinking habits, overweightness, carotid stenosis and HHCY.

directly influence LAA susceptibility and this effect may be independent of traditional risk factors for IS. Individuals carrying the T-allele exhibit an increased susceptibility to LAA. Furthermore, multifactorial logistic regression analysis revealed an interaction between rs2048894 and rs5343. Additionally, we confirmed that inflammation levels differed among patients with different EDNRA gene polymorphisms compared to controls.

In this study, we selected NLRP3, CCL18, IL-6, IL-8, IL-10, and IL-18 to analyze their roles in mediating the effect of EDNRA on susceptibility to LAA. Previous studies have demonstrated an interaction between

Model[*]	Training bal. acc.	Testing bal. acc.	Sign test (P value)	CVC
12	0.5425	0.5138	7(0.1719)	6/10
12,16	0.5654	0.5432	9(0.0107)	10/10
9,15,16	0.5830	0.4977	6(0.3730)	4/10
5,13,15,16	0.6061	0.4795	4(0.8281)	2/10
6,9,13,15,16	0.6258	0.4776	3(0.9453)	3/10
1,6,8,12,14,16	0.6458	0.4892	5(0.6230)	4/10
1,6,8,9,13,14,16	0.6599	0.4999	6(0.3770)	6/10
1,6,8,9,12,14,15,16	0.6718	0.4841	5(0.6230)	5/10
1,6,8,9,10,12,14,15,16	0.6783	0.4861	4(0.8281)	5/10
1,6,8,9,10,11,12,14,15,16	0.6812	0.4729	3(0.9453)	5/10
1,6,7,8,9,10,11,12,14,15,16	0.6833	0.4764	4(0.8281)	2/10
1,6,7,8,9,10,11,12,13,14,15,16	0.6849	0.4763	5(0.6230)	6/10
1,5,6,7,8,9,10,11,12,13,14,15,16	0.6860	0.4791	4(0.8281)	9/10
1,2,5,6,7,8,9,10,11,12,13,14,15,16	0.6860	0.4768	3(0.9453)	9/10
1,2,3,5,6,7,8,9,10,11,12,13,14,15,16	0.6860	0.4768	3(0.9453)	9/10
1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16	0.6860	0.4758	3(0.9453)	10/10

Table 7. GMDR model of gene interaction. Notes: [*]The numbers 1–16 represent rs1878406, rs6842241, rs6841581, rs1801708, rs10305863, rs702757, rs7657903, rs10305895, rs908581, rs7655892, rs78047355, rs2048894, rs5333, rs5335, rs5342, rs5343. CVC, cross-validation consistency.

inflammatory factors, calcium ion signaling pathways, and the EDNRA gene[45]. In patients with systemic sclerosis, the EDNRA gene has been found to participate in the activation of inflammatory factors such as interleukin-8 (IL-8) and chemokine CC-motif ligand 18 (CCL18)[46], highlighting the regulatory role of the EDNRA/inflammatory factor pathway in the inflammatory response[47]. Additionally, NLRP3 acts as a platform for activating caspase-1, which subsequently induces the release of pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18[48]. These findings suggested that the cascade-like interactions among the EDNRA gene, inflammatory factors, and NLRP3 activation might play a role in disease pathogenesis.

By examining the levels of these inflammatory factors, we found that in patients with LAA, genetic polymorphisms at multiple loci of the EDNRA gene were found to be associated with levels of inflammation-related factors, including NLRP3, IL-6, IL-8, IL-10 and IL-18. Furthermore, a positive correlation was observed between EDNRA expression levels and those of both NLRP3 and IL-18 in LAA patients. Previous studies have shown that after stroke, the ischemic cascade response releases large amounts of IL-6[49], and CD4＋T cells produce more IL-10[50]. In addition, IL-18 has a pro-atherosclerotic profile, and increased serum IL-18 in patients with coronary artery disease has been shown to play a role in the process of brain cell injury[51]. The NLRP3 inflammasome has been identified as a key component in the intervention of inflammatory factors in the post-ischemic period. plays an important role[52]. Our study suggests that inflammatory mechanisms may mediate the association of genetic polymorphisms in EDNRA with the pathogenesis of LAA.

Previous studies have examined the association between EDNRA single locus and IS in Asian and European populations; however, the findings have yielded inconsistent results[11,23,48,53,54]. Given that stroke exhibits distinct characteristics across different geographic regions[3], investigating genetic polymorphisms and LAA risk in specific populations will facilitate the development of more precise treatment strategies for LAA. Moreover, the aforementioned studies solely investigated a single genetic locus, and currently, there exists an absence of a comprehensive analysis regarding the potential association and underlying mechanisms between the genetic polymorphism of EDNRA and the risk of LAA. In this study, patients and controls were exclusively recruited from the Han population of the Third People's Hospital of Chengdu. We conducted a comprehensive analysis on the correlation between 16 tagSNPs polymorphisms in the EDNRA and susceptibility to LAA, revealing a potential link to an inflammatory response. The implementation of these initiatives will facilitate the development of precise strategies for preventing and controlling LAA, specifically targeting distinct populations.

The results indicate a strong association between the rs5343 genetic polymorphism and LAA pathogenesis. Of the various inflammatory factors examined, only IL-18 demonstrated a significant correlation with the rs5343 genetic polymorphism. IL-18 belongs to the IL-1 family of cytokines, which comprises 11 cytokines that stimulate innate immune system activity[55,56]. IL-18 has the ability to activate both innate and adaptive immune responses, exhibiting pleiotropic effects in various cytokine environments. This highlights its crucial pathophysiological role in maintaining health and contributing to disease progression[57,58]. Specifically, compelling evidence links IL-18 to IS. During the early phase, neuronal cells primarily express IL-18, while microglia express it during later stages. Stroke-induced inflammation is associated with IL-18, and initial serum levels of this cytokine may predict stroke outcomes[59]. However, further investigation is necessary to determine whether rs5343 affects LAA pathogenesis through immune response and IL-18.

rs5343, also referred to as rs17475065 or rs60414981, is situated within the 3'-untranslated region (3'UTR) of the EDNRA gene. The noncoding regions of mRNAs, known as 3'UTRs, play a crucial role in regulating protein levels by controlling mRNA stability and translation through AU-rich elements and miRNAs[60–62]. Additionally, 3'UTRs facilitate local translation by regulating mRNA localization[63,64]. Numerous prior studies have
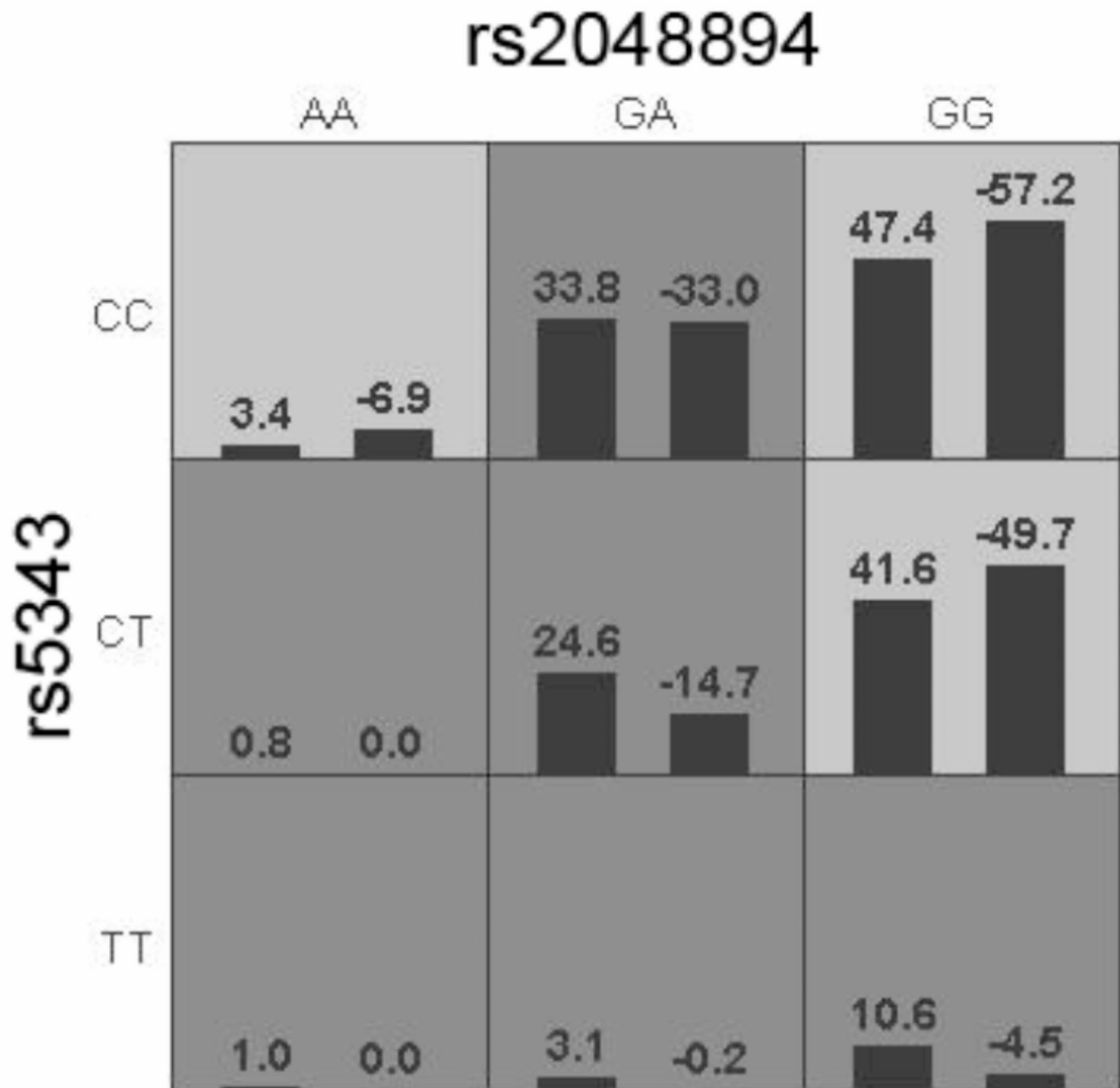
Fig. 2. Risk of 9 different combinations of rs2048894 and rs5343 genotypes. High-risk cells are indicated by dark color, low-risk cells are indicated by light color. The high-risk interaction genotype was assigned as one, and low-risk interaction genotype was assigned as zero in multivariable logistic regression analysis.

demonstrated the association between stroke pathogenesis and 3'UTRs of diverse genes. Lu et al. demonstrated that the SNP rs8679 located in the 3'UTR of PARP1 may serve as a protective factor for patients with IS[65]. Kim et al. suggested that Thymidylate Synthase 3'UTR variants were linked to the susceptibility of IS and silent brain infarction[66]. Additionally, they discovered that MTHFR 3'UTR polymorphisms contribute to the pathogenesis of IS. The combined effects of MTHFR 3'UTR polymorphisms and tHcy/folate levels may play a role in the prevalence of stroke[67]. Furthermore, it has been demonstrated that certain 3'UTRs are associated with the risk of stroke pathogenesis through miRNA regulation[68,69]. This has inspired us to further investigate the mechanism by which rs5343 affects the pathogenesis of LAA.

There may be some limitations to this study. Firstly, it was a single-center case-control study with patients from the same region and ethnicity, so there may be some bias in gene expression. Secondly, despite the sample size being relatively large, there was still some heterogeneity in the traditional risk factors of the patients enrolled in this study after adjusting for the baseline levels. Although these risk factors were independent of each other, did not interact with the genes we studied and did not affect the primary outcome of this study, future studies should try to minimize these effects. In addition, the EDNRA gene was identified in this study as possibly
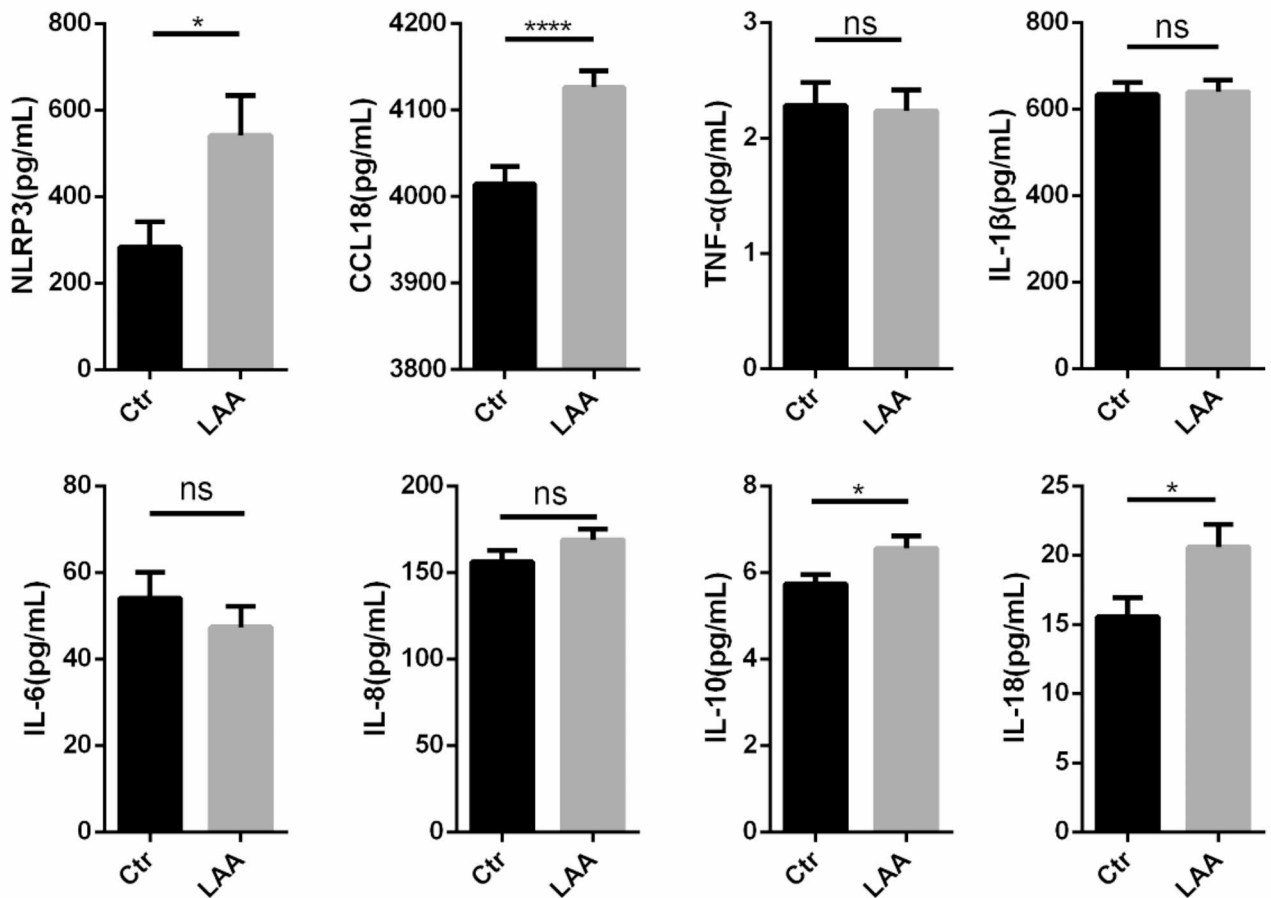
Fig. 3. Levels of inflammation-related factors in the control group and LAA group. Outlier detection was conducted using the 3-sigma criterion. Values are shown as means ± SEM, $^*p < 0.05$, $^{****}p < 0.0001$.

influencing LAA susceptibility by affecting inflammatory factors such as IL-18, but the mechanism was not elucidated, and further studies are needed to explore the mechanism in the future.

Conclusion

This study provides strong evidence that the rs5343 TT genotype of the EDNRA gene is significantly associated with an increased risk of LAA. LAA cases exhibit higher levels of NLRP3, IL-10, IL-18, and CCL-18, with a significant relationship between EDNRA polymorphisms and these inflammatory markers. The findings suggest that EDNRA may contribute to LAA susceptibility through the NLRP3-mediated inflammatory pathway. These insights provide valuable directions for future research and potential strategies for the prevention and management of LAA.
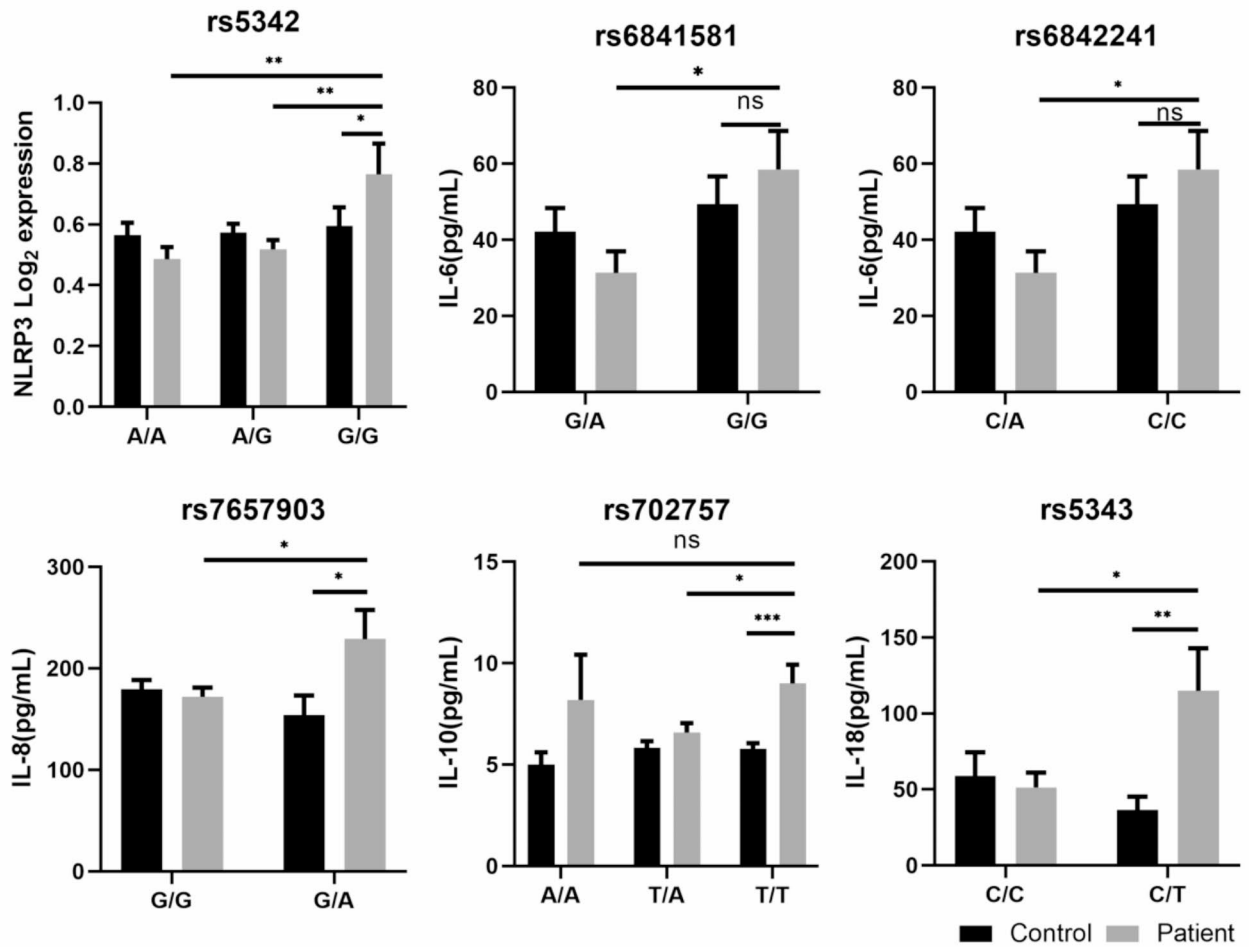
Fig. 4. EDNRA polymorphism is associated with inflammation in LAA patients. No significant differences were observed in the levels of inflammatory factors at the relevant polymorphic loci in the control group. Values are shown as means $\pm$ SEM, $^*p < 0.05$, $^{**}p < 0.01$.
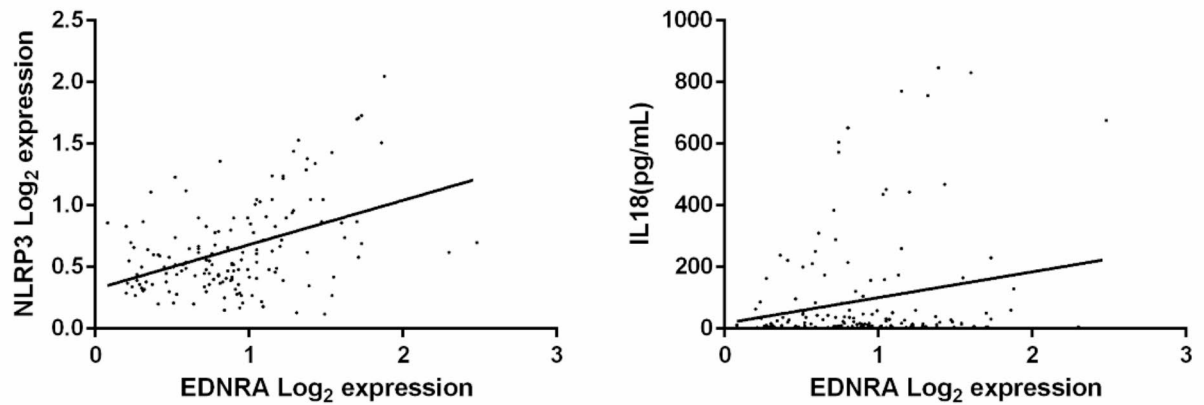

Fig. 5. EDNRA expression is positively correlated with NLRP3 expression and IL18 levels in LAA patients.

GENE	Group	Pearson's *R*	*P*
NLRP3	LAA	0.437	< 0.001
	Ctr	0.243	0.002
CCL18	LAA	-0.041	0.611
	Ctr	-0.070	0.376
IL-6	LAA	0.061	0.454
	Ctr	-0.021	0.792
IL-8	LAA	0.092	0.255
	Ctr	0.052	0.515
IL-10	LAA	0.004	0.958
	Ctr	-0.042	0.600
IL-18	LAA	0.212	0.009
	Ctr	-0.052	0.515

Table 8. Description of the association between EDNRA and diverse levels of inflammatory factors. Notes: LAA, large artery atherosclerotic stroke; Ctr, Control.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Collaborators, G. B. D. S. Global, regional, and national burden of stroke and its risk factors, 1990–2019: A systematic analysis for the global burden of Disease Study 2019. *Lancet Neurol.* **20**(10), 795–820 (2021).
2. Tsao, C. W. et al. Heart disease and stroke statistics-2023 update: A Report from the American Heart Association. *Circulation* **147**(8), e93–e621 (2023).
3. Ma, Q. et al. Temporal trend and attributable risk factors of stroke burden in China, 1990–2019: An analysis for the global burden of disease study 2019. *Lancet Public. Health* **6**(12), e897–e906 (2021).
4. Ornello, R. et al. Distribution and temporal trends from 1993 to 2015 of ischemic stroke subtypes: A systematic review and meta-analysis. *Stroke* **49**(4), 814–819 (2018).
5. Diener, H. C. & Hankey, G. J. Primary and secondary prevention of ischemic stroke and cerebral hemorrhage: JACC focus seminar. *J. Am. Coll. Cardiol.* **75**(15), 1804–1818 (2020).
6. Boehme, A. K., Esenwa, C. & Elkind, M. S. Stroke risk factors, genetics, and prevention. *Circ. Res.* **120**(3), 472–495 (2017).
7. Xu, J. et al. Trends and Risk factors Associated with Stroke recurrence in China, 2007–2018. *JAMA Netw. Open.* **5**(6), e2216341 (2022).
8. Abbott, A. Asymptomatic carotid stenosis and stroke risk. *Lancet Neurol.* **20**(9), 698–699 (2021).
9. Tondel, B. G., Morelli, V. M., Hansen, J. B. & Braekkan, S. K. Risk factors and predictors for venous thromboembolism in people with ischemic stroke: A systematic review. *J. Thromb. Haemost* **20**(10), 2173–2186 (2022).
10. Dichgans, M., Pulit, S. L. & Rosand, J. Stroke genetics: Discovery, biology, and clinical applications. *Lancet Neurol.* **18**(6), 587–599 (2019).
11. Malik, R. et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat. Genet.* **50**(4), 524–537 (2018).
12. Neurology Working Group of the Cohorts for H, Aging Research in Genomic Epidemiology Consortium tSGN, the International Stroke Genetics C. Identification of additional risk loci for stroke and small vessel disease: A meta-analysis of genome-wide association studies. *Lancet Neurol.* **15**(7), 695–707 (2016).
13. Yu, J. C., Pickard, J. D. & Davenport, A. P. Endothelin ETA receptor expression in human cerebrovascular smooth muscle cells. *Br. J. Pharmacol.* **116**(5), 2441–2446 (1995).
14. Yuan, W. et al. Endothelin-receptor antagonist can reduce blood pressure in patients with hypertension: A meta-analysis. *Blood Press.* **26**(3), 139–149 (2017).
15. Pope, J. E. et al. State-of-the-art evidence in the treatment of systemic sclerosis. *Nat. Rev. Rheumatol.* **19**(4), 212–226 (2023).
16. Dai, D. Z. & Dai, Y. Role of endothelin receptor A and NADPH oxidase in vascular abnormalities. *Vasc. Health Risk Manag.* **6**, 787–794 (2010).
17. Li, T. C. et al. Associations of EDNRA and EDN1 polymorphisms with carotid intima media thickness through interactions with gender, regular exercise, and obesity in subjects in Taiwan: Taichung Community Health Study (TCHS). *Biomed. (Taipei)* **5**(2), 8 (2015).
18. Bis, J. C. et al. Meta-analysis of genome-wide association studies from the CHARGE consortium identifies common variants associated with carotid intima media thickness and plaque. *Nat. Genet.* **43**(10), 940–947 (2011).
19. Hemerich, D. et al. Impact of carotid atherosclerosis loci on cardiovascular events. *Atherosclerosis* **243**(2), 466–468 (2015).
20. Levin, E. R. Endothelins. *N. Engl. J. Med.* **333**(6), 356–363 (1995).
21. Ehrenreich, H. et al. Endothelins, peptides with potent vasoactive properties, are produced by human macrophages. *J. Exp. Med.* **172**(6), 1741–1748 (1990).
22. Bjorkegren, J. L. M. & Lusis, A. J. Atherosclerosis: Recent developments. *Cell* **185**(10), 1630–1645 (2022).
23. Wei, W. et al. EDNRA gene rs1878406 polymorphism is associated with susceptibility to large artery atherosclerotic stroke. *Front. Genet.* **12**, 783074 (2021).
24. Gao, S., Wang, Y. J., Xu, A. D., Li, Y. S. & Wang, D. Z. Chinese ischemic stroke subclassification. *Front. Neurol.* **2**, 6 (2011).
25. Kelly, T. N. et al. Cigarette smoking and risk of stroke in the Chinese adult population. *Stroke* **39**(6), 1688–1693 (2008).
26. Guideline for the pharmacological treatment of hypertension in adults. WHO Guidelines App roved by the Guidelines Review Committee. Geneva (2021).

27. Unger, T. et al. 2020 International Society of Hypertension global hypertension practice guidelines. *Hypertension* **75**(6), 1334–1357 (2020).
28. Alberti, K. G. & Zimmet, P. Z. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet. Med.* **15**(7), 539–553 (1998).
29. Joint committee issued Chinese guideline for the management of dyslipidemia in a. 2016 Chinese guideline for the management of dyslipidemia in adults. *Zhonghua Xin Xue Guan Bing Za Zhi* **44**(10), 833–853 (2016).
30. Chen, C., Lu, F. C. & Department of Disease Control Ministry of Health PRC. The guidelines for prevention and control of overweight and obesity in Chinese adults. *Biomed. Environ. Sci.* **17 Suppl**, 1–36 (2004).
31. Liu, L. S. & Writing Group of Chinese Guidelines for the Management of H. 2010 Chinese guidelines for the management of hypertension. *Zhonghua Xin Xue Guan Bing Za Zhi* **39**(7), 579–615 (2011).
32. North American Symptomatic Carotid Endarterectomy Trial. Methods, patient characteristics, and progress. *Stroke* **22**(6), 711–720 (1991).
33. Jiang, H. et al. Peripheral blood mitochondrial DNA content, A10398G polymorphism, and risk of breast cancer in a Han Chinese population. *Cancer Sci.* **105**(6), 639–645 (2014).
34. Wei, J. et al. MiR-196a2 rs11614913 T > C polymorphism and risk of esophageal cancer in a Chinese population. *Hum. Immunol.* **74**(9), 1199–1205 (2013).
35. Xiao, C-L. et al. N6-methyladenine DNA modification in the human genome. *Mol. Cell* **71**(2), 306–318 (2018).
36. Pei, X. et al. circMET promotes NSCLC cell proliferation, metastasis, and immune evasion by regulating the miR-145-5p/CXCL3 axis. *Aging (Albany NY)* **12**(13), 13038 (2020).
37. Thakkinstian, A., McElduff, P., D'Este, C., Duffy, D. & Attia, J. A method for meta-analysis of molecular association studies. *Stat. Med.* **24**(9), 1291–1306 (2005).
38. Dudoit, S., Yang, Y. H., Callow, M. J. & Speed, T. P. Statistical methods for identifying differentially expressed genes in replicated cDNA microarray experiments. *Stat. Sin.* 111–139 (2002).
39. Ritchie, M. D., Hahn, L. W. & Moore, J. H. Power of multifactor dimensionality reduction for detecting gene-gene interactions in the presence of genotyping error, missing data, phenocopy, and genetic heterogeneity. *Genet. Epidemiol.* **24**(2), 150–157 (2003).
40. Yi, X. et al. Interactions among variants in P53 apoptotic pathway genes are associated with neurologic deterioration and functional outcome after acute ischemic stroke. *Brain Behav.* **11**(5), e01492 (2021).
41. Lutgens, E. et al. Immunotherapy for cardiovascular disease. *Eur. Heart J.* **40**(48), 3937–3946 (2019).
42. Weber, C. & Noels, H. Atherosclerosis: Current pathogenesis and therapeutic options. *Nat. Med.* **17**(11), 1410–1422 (2011).
43. Kaptoge, S. et al. Inflammatory cytokines and risk of coronary heart disease: New prospective study and updated meta-analysis. *Eur. Heart J.* **35**(9), 578–589 (2014).
44. Papadopoulos, A. et al. Circulating Interleukin-6 levels and incident ischemic stroke: A systematic review and Meta-analysis of prospective studies. *Neurology* **98**(10), e1002–e12 (2022).
45. Hamby, M. E. et al. Inflammatory mediators alter the astrocyte transcriptome and calcium signaling elicited by multiple G-protein-coupled receptors. *J. Neurosci.* **32**(42), 14489–14510 (2012).
46. Günther, J. et al. Angiotensin receptor type 1 and endothelin receptor type A on immune cells mediate migration and the expression of IL-8 and CCL18 when stimulated by autoantibodies from systemic sclerosis patients. *Arthritis Res. Ther.* **16**(2), R65 (2014).
47. Dinarello, C. A. Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* **27**, 519–550 (2009).
48. Yamaguchi, S. et al. Genetic risk for atherothrombotic cerebral infarction in individuals stratified by sex or conventional risk factors for atherosclerosis. *Int. J. Mol. Med.* **18**(5), 871–883 (2006).
49. Esenwa, C. C. & Elkind, M. S. Inflammatory risk factors, biomarkers and associated therapy in ischaemic stroke. *Nat. Rev. Neurol.* **12**(10), 594–604 (2016).
50. DeLong, J. H., Ohashi, S. N., O'Connor, K. C. & Sansing, L. H. Inflammatory responses after ischemic stroke. *Semin Immunopathol.* **44**(5), 625–648 (2022).
51. Kaplanski, G. Interleukin-18: Biological properties and role in disease pathogenesis. *Immunol. Rev.* **281**(1), 138–153 (2018).
52. Wanderer, A. A. Ischemic-reperfusion syndromes: Biochemical and immunologic rationale for IL-1 targeted therapy. *Clin. Immunol.* **128**(2), 127–132 (2008).
53. Zhang, L. & Sui, R. Effect of SNP polymorphisms of EDN1, EDNRA, and EDNRB gene on ischemic stroke. *Cell. Biochem. Biophys.* **70**(1), 233–239 (2014).
54. Dubovyk, Y. I., Oleshko, T. B., Harbuzova, V. Y. & Ataman, A. V. Positive association between EDN1 rs5370 (Lys198Asn) polymorphism and large artery stroke in a Ukrainian population. *Dis. Markers* **2018**, 1695782 (2018).
55. Sims, J. E. & Smith, D. E. The IL-1 family: Regulators of immunity. *Nat. Rev. Immunol.* **10**(2), 89–102 (2010).
56. Sims, J. E. et al. A new nomenclature for IL-1-family genes. *Trends Immunol.* **22**(10), 536–537 (2001).
57. Nakanishi, K. Unique action of interleukin-18 on T cells and other immune cells. *Front. Immunol.* **9**, 763 (2018).
58. Yasuda, K., Nakanishi, K. & Tsutsui, H. Interleukin-18 in health and disease. *Int. J. Mol. Sci.* **20**(3). (2019).
59. Zhu, H. et al. Interleukins and ischemic stroke. *Front. Immunol.* **13**, 828447 (2022).
60. Barreau, C., Paillard, L. & Osborne, H. B. AU-rich elements and associated factors: Are there unifying principles? *Nucleic Acids Res.* **33**(22), 7138–7150 (2005).
61. Bartel, D. P. MicroRNAs: Target recognition and regulatory functions. *Cell* **136**(2), 215–233 (2009).
62. Chen, C. Y. & Shyu, A. B. AU-rich elements: Characterization and importance in mRNA degradation. *Trends Biochem. Sci.* **20**(11), 465–470 (1995).
63. Martin, K. C. & Ephrussi, A. mRNA localization: Gene expression in the spatial dimension. *Cell* **136**(4), 719–730 (2009).
64. Niedner, A., Edelmann, F. T. & Niessing, D. Of social molecules: The interactive assembly of ASH1 mRNA-transport complexes in yeast. *RNA Biol.* **11**(8), 998–1009 (2014).
65. Gu, L., Wang, Q., Xu, G. & Liu, D. Functional genetic variation in 3'UTR of PARP1 indicates a decreased risk and a better severity of ischemic stroke. *Int. J. Neurosci.* 1–6 (2022).
66. Kim, J. O. et al. The 3'-UTR polymorphisms in the Thymidylate synthase (TS) Gene Associated with the risk of ischemic stroke and silent brain infarction. *J. Pers. Med.* **11**(3), 200 (2021).
67. Kim, J. O. et al. Interplay between 3'-UTR polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene and the risk of ischemic stroke. *Sci. Rep.* **7**(1), 12464 (2017).
68. Chen, J. et al. A functional variant in the 3'-UTR of angiopoietin-1 might reduce stroke risk by interfering with the binding efficiency of microRNA 211. *Hum. Mol. Genet.* **19**(12), 2524–2533 (2010).
69. Lu, J., Xu, F. & Lu, H. LncRNA PVT1 regulates ferroptosis through miR-214-mediated TFR1 and p53. *Life Sci.* **260**, 118305 (2020).

Author contributions

Declarations

Competing interests

The authors declare no competing interests.
The authors declare no conflict of interest.

Additional information

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