Feng *et al. Artery Research* 2024, **30**:5
https://doi.org/10.1007/s44200-024-00051-x

Artery Research

RESEARCH ARTICLE

Open Access

LncRNA HOTAIR: A Novel Biomarker for the Diagnosis of Asymptomatic Carotid Artery Stenosis and Prediction of the Onset of Cerebral Ischemic Events

Pengcheng Feng[1†], Chenguang Tong[2†], Yuan Li[3] and Li Liu[3*]

Abstract

Background Carotid artery stenosis (CAS) is a major cause of cerebral ischemic events (CIE). The purpose of the research was to reveal the diagnostic accuracy of long non-coding RNA hox transcript antisense intergenic RNA (HOTAIR) in asymptomatic carotid artery stenosis (ACAS) patients and its predictive significance in CIE incidence.

Methods 88 patients with ACAS and 80 controls were included. Blood samples were collected and serum HOTAIR levels were detected by qRT-PCR. Logistic regression examined factors associated with the degree of carotid stenosis. The receiver operating characteristic (ROC) curve assessed the diagnostic accuracy of HOTAIR in identifying patients with ACAS. Predictive value of serum HOTAIR levels for the occurrence of CIE was assessed by Kaplan–Meier curves and Cox regression.

Results Serum HOTAIR was markedly lower in ACAS patients than in controls ($P<0.001$). Logistic regressions confirmed that HOTAIR levels correlated with severe carotid artery stenosis (OR = 0.289, 95% CI = 0.107–0.786, $P=0.015$). ROC's AUC was 0.925, indicating high sensitivity and specificity in differentiating between the controls and patients with ACAS. Furthermore, CIE-positive patients had lower HOTAIR levels than CIE-negative, and the degree of carotid stenosis (HR = 4.566, 95% CI = 1.206–17.292, $P=0.025$) and HOTAIR levels (HR = 0.244, 95% CI = 0.072–0.824, $P=0.023$) were independent risk factors for the development of CIE. Patients with lower HOTAIR were more susceptible to CIE (log-rank $P=0.001$).

Conclusions Serum HOTAIR was reduced in patients with ACAS and may be a non-invasive diagnostic biomarker for ACAS and predicts the development of CIE.

Keywords Carotid artery stenosis, HOTAIR, CIE, Biomarker

†Pengcheng Feng and Chenguang Tong contributed equally to this study.

*Correspondence:
Li Liu
liulipzh1@163.com
Full list of author information is available at the end of the article

1 Background

Cerebral ischemic events (CIE) are the major cause of long-term disability and the fifth leading cause of death [1]. Carotid artery stenosis (CAS) is a primary risk factor for the development of CIE, accounting for 20% of cases [2], and refers to a pathological condition in which atheromatous plaque accumulates in the arterial wall, causing narrowing or constriction of the inner surface of the vessel and thus blocking blood flow [3, 4]. The progression of CAS is usually insidious, and patients may remain asymptomatic until the onset of transient ischemic attack or stroke appears, thus missing the best time for management [5]. Although imaging methods are used for the diagnosis and screening of asymptomatic CAS (ACAS), they have different drawbacks, such as the high price is not suitable for universal screening [6, 7]. Therefore, effective markers for diagnosing ACAS and predicting the incidence of CIE can draw the attention of patients and clinicians, and provide early nursing care and intervention to prevent the occurrence of CIE. Previous studies have analyzed the future occurrence of CIE by regression based on common risk factors such as age, gender, body mass index (BMI), fasting blood glucose (FBG), total cholesterol (TC), and so on [8].

Long non-coding RNAs (LncRNAs) are RNA molecules longer than 200 nt that lack protein-coding capabilities and play critical regulatory roles in disease physiology and pathology, and their dysregulation can be employed as diagnostic and prognostic biomarkers. Hox transcript antisense intergenic RNA (HOTAIR) is a 2.2-kd long LncRNA molecule located on chromosome 12q13.13, encoded in the *HOXC* gene cluster, and transcribed by RNA polymerase [9]. HOTAIR has been widely reported as an oncogene [10], and its role in cardiovascular and cerebrovascular diseases has also drawn attention. For example, HOTAIR is a myocardial infarction (MI) protector in rats by eliminating miR-519d-3p [11]. HOTAIR dysregulation is involved in neurological impairment in ischemic stroke [12]. HOTAIR modulates the progression of Parkinson's disease [13] and alleviates cerebral ischemia–reperfusion injury [14]. At the same time, dysregulated HOTAIR was also involved in cardiac hypertrophy [15], heart failure [16], acute myocardial infarction (AMI) [17], and coronary artery diseases [18]. Notably, atherosclerosis (AS) is central to the pathogenesis of CAS, HOTAIR is reduced in endothelial cells of atherosclerotic plaques [19], and overexpression of HOTAIR alleviates AS by reducing inflammatory factors [20]. The present evidence suggests a strong association between HOTAIR and CAS, however, the clinical implication of HOTAIR in CAS has not been reported.

Hence, serum samples were collected to examine the expression of HOTAIR in patients of ACAS and controls. At the same time, the diagnostic significance of HOTAIR for ACAS patients and the predictive significance for the development of CIE were also evaluated.

2 Methods

2.1 Research Participant

The present research was designed as a retrospective cohort study, which was conducted with the approval of the Affiliated Hospital of Panzhihua University Ethics Committee. Subjects participated voluntarily, and their written informed consent was obtained before blood collection. The experimental procedures were conducted following the Declaration of Helsinki and its modification. The inclusion criteria for patients with ACAS follow (1) adults (18–80 years old); (2) first finding of greater than 50% ipsilateral stenosis; (3) no history of ischemic stroke (IS, according to the National Institute of Health Stroke Scale table), transient ischemic attack (TIA), or amaurosis. Patients with diabetes, acute or chronic inflammation, and malignancies, as well as those taking statins, were also excluded. In addition, healthy individuals who underwent health checkups in the same period were included as the controls. They were age (mean age:65 years, SD = 11; range from 18 to 80 years)- and gender (40 male and 48 female)-matched to the patients, with carotid artery stenosis less than 20% and clinical indicators were normal, no history of cardiovascular or cerebrovascular diseases, infections, tumors, or carotid artery surgery.

A total of 88 patients with ACAS (mean age 65 ± 11 years; 40 male, female 48) and 80 Controls (mean age 64 ± 10 years; 37 males, 43 female) were admitted from June 2015 and December 2016 in the Affiliated Hospital of Panzhihua University (Since the present study is a retrospective cohort, the sample size was limited to several eligible patients and controls during this period). Doppler ultrasound was performed by the same investigator using the same ultrasound equipment to measure the degree of carotid stenosis (mild: < 50% stenosis, moderate: 50–69% stenosis, severe: 70–94% stenosis) according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) [21, 22]. The demographic and biochemical indicators of ACAS patients and controls were recorded in Table 1.

2.2 Sample Collection

Blood samples (10 ml) from ACAS patients and controls were collected in tubes and centrifuged at 2000*g* for 10 min at 4 ℃ within 1 h. The supernatant was removed and stored at – 80 ℃ until use.

Table 1 Statistical analysis of basic clinical information of all subjects

Characteristic	ACAS patients $N=88$	Controls $N=80$	*P* value
Age (years)	65 ± 11	64 ± 10	0.598
Gender (male/female)	40/48	37/43	0.918
BMI (kg/m [2])	23.71 ± 5.51	22.38 ± 4.43	0.089
FBG (mg/dL)	96.58 ± 14.86	92.56 ± 18.02	0.116
TC (mg/dL)	193.33 ± 5.16	192.21 ± 3.69	0.109
TG (mg/dL)	125.11 ± 14.31	121.30 ± 12.65	0.070
HDL (mg/dL)	48.26 ± 3.52	49.01 ± 3.94	0.192
LDL (mg/dL)	113.60 ± 5.65	112.78 ± 6.26	0.374
SBP (mm Hg)	131.64 ± 11.94**	126.05 ± 13.68	**0.003**
DBP (mm Hg)	83.70 ± 10.56**	77.33 ± 16.30	**< 0.001**
CRP (mg/l)	24.51 ± 3.56***	4.61 ± 1.98	**< 0.001**

Instructions: *CAS* carotid artery stenosis, *BMI* body mass index, *FBG* fasting blood glucose, *TC* total cholesterol, *TG* triglycerides, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *CRP* C-reactive protein. Data are expressed as n or mean ± standard deviation. $^{**}P < 0.01$, $^{***}P < 0.001$, Controls compared with patients with ACAS

2.3 Follow-up Survey

ACAS patients were followed up for 5 years by telephone or readmission every 3 or 6 months, and the information on TIA, ischemic stroke (IS), blackout, or sudden death (due to IS) was recorded. Where IS was described as a new focal neurological deficit lasting more than 24 h, or brain imaging by magnetic resonance imaging (MRI) or computed tomography (CT), diagnosed as acute cerebral infarction. TIA was described as a new neurological deficit with transient focal brain dysfunction consistent with a duration of less than 24 h but without cerebral infarction on imaging [23]. Their diagnoses are confirmed by neurologists to ensure reliability. The follow-up study lasted a long time, and data from 4 patients were completely lost during follow-up due to changing phone numbers, but the loss rate was < 5%. At the same time, a conservative approach was used for further analysis, and the lost participants were considered non-CIE individuals. The process is outlined in the schematic diagram of participant flow (Supplementary file 2).

2.4 Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

Total RNA was first extracted from the subject's serum sample. Briefly, 100 µL of serum was lysed in TRIzol LS (Invitrogen, Carlsbad, CA, USA), incubated at room temperature for 5 min, chloroform was added, the aqueous phase was removed, 100% ethanol was added, the column was passed through the adsorption column several times, and finally 50 µL of RNase-free water was used for solubilization. The quality and concentration of RNA were subsequently assessed using the NanoDrop 2000 spectrophotometer (OD260/OD280 = 1.8–2.0; concentrations ≥ 250 ng /µL were required). When its value met 1.8–2.0, 1 µg of RNA was reversed and transcribed into cDNA with the help of Fasting gDNA dispelling RT SuperMix Kit (Tiangen Biotech, Beijing, China). Then, 2 µl of cDNA was mixed with the reagents in SuperReal PreMix Plus (SYBR Green) (Tiangen Biotech, Beijing, China) as a template, and primers for HOTAIR and internal reference GAPDH were added. qRT-PCR reactions were carried out in an Applied Biosystems 7500 real-time PCR system. Primer sequences are as follows: HOTAIR forward, 5'-CAGTGGGGAACTCTGACTCG-3', and reverse 5'-GTGCCTGGTGCTCTCTTACC-3', and GAPDH forward, 5'- TGAAGGTCGGAGTCAACGGAT TTGGT-3', and reverse, 5'-CATGTGGGCCATGAGGTC CACCAC-3', and synthesized by Genepharma (Suzhou, China). Each experiment was repeated three times and the relative expression levels of serum HOTAIR were calculated by the $2^{-\Delta\Delta Ct}$ methods based on threshold cycle (CT) for the target gene HOTAIR and the reference gene GAPDH ($\Delta Ct = Ct_{(HOTAIR)} - Ct_{(GAPDH)}$, $\Delta\Delta Ct = \Delta Ct_{(patients\ group)} - \Delta Ct_{(control\ group)}$) [24].

2.5 Statistical Analysis

Data were displayed as mean ± standard deviation and tested in triplicate. ROC was performed to evaluate the diagnostic accuracy of serum HOTAIR levels in distinguishing ACAS patients from controls. ACAS and control groups were used as independent dichotomous variables, and HOTAIR, gender, age, BMI, FBG, and other clinical indicators as independent variables (where the mean was used as the cut-off value, measures greater than the mean were defined as 1, and measures less than the mean were defined as 0, and categorical variables, such as gender, were defined as 0 in male and 1 in female), and factors with significance levels of $P < 0.05$ in univariate logistic analyses were selected for inclusion in the logistic regression analysis models of risk factors independently associated with the degree of stenosis, and adjusted advantage ratio (OR) and 95% confidence intervals (95% CI). Kaplan–Meier (K-M) curves and COX regression were conducted to investigate the incidence of CIE in patients with different levels of HOTAIR. Univariate COX analysis was performed for univariate covariates of potential risk factors (including age, gender, BMI, FBG, TC, TG, HDL, LDL, SBP, DBP, CPR, and HOTAIR), and covariates with $P < 0.05$ in the initial univariate analyses were selected for a multivariable COX proportional risk modeling, using Scaled Schienfeld residuals for risk assumptions for the variables, and 95%CI were used to characterize variable distributions.

Statistically, differences between groups were compared using a two-tailed Student's t-test, and the levels of statistical significance of the P-value were taken to be less than 0.05. SPSS 23.0 and GraphPad Prism for clinical data processing and graphing. To conduct this study, guidelines for reporting prediction models and validation studies in Health Research [Transparent Reporting of a multivariable prediction model for individual prognosis or Diagnosis (the TRIPOD statement)] were followed [25]. The TRIPOD checklist is presented in Supplementary File 1.

3 Results

3.1 Comparison of Baseline Demographic and Laboratory Characteristics

Table 1 reveals the baseline clinical information of 88 ACAS patients and 80 controls. No statistical differences were found between ACAS patients and controls in age, gender, BMI, total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, and fasting blood glucose ($P > 0.05$), but systolic blood pressure, diastolic blood pressure, and C-reactive protein were higher in ACAS patients than controls ($P < 0.05$).

3.2 The Expression Levels of lncRNA HOTAIR in the Serum of ACAS Patients

Subsequently, serum HOTAIR levels of ACAS patients and controls were measured via qRT-PCR. Figure 1A revealed that serum HOTAIR in ACAS patients (0.53 ± 0.19) was reduced than that in controls (1.00 ± 0.25, $P < 0.001$, Fig. 1A).

3.3 Serum lncRNA HOTAIR is Associated with Severe Carotid Stenosis

The degree of carotid stenosis is an essential indicator of ACAS care and treatment [26]. For this reason, we further explored the relationship between the levels of HOTAIR and clinical data with the degree of carotid stenosis. Patients with ACAS were divided into the moderate carotid stenosis group ($n = 47$) and severe carotid stenosis group ($n = 41$) according to the degree of carotid stenosis [27]. Univariate logistic regression analysis and Multivariable logistic regression revealed that serum HOTAIR levels (OR = 0.289, 95% CI = 0.107–0.786, $P = 0.015$, Table 2) correlate with the degree of ACAS severe stenosis.

3.4 Serum LncRNA HOTAIR can Serve as a Diagnostic Biomarker for ACAS

Later, we examined whether serum HOTAIR has potential as a diagnostic biomarker for ACAS. The overall model performance was good, indicated by an AUC of 0.925 (95% CI = 0.882–0.969), and scaled Brier score of 0.102 (Supplemental table), and the best sensitivity and specificity were 83.0% and 90.0%, respectively, when the cut-off value was 0.743, which significantly identified ACAS patients from the controls (95% CI = 0.882–0.969, Fig. 1B). In summary, our data confirm that HOTAIR is a feasible diagnostic biomarker for ACAS.

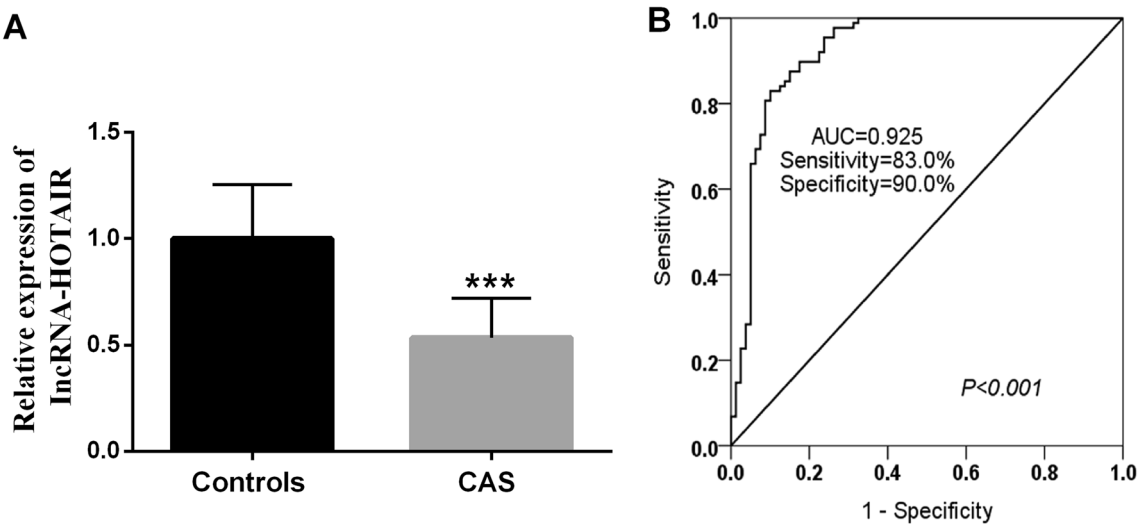

Fig. 1 The levels of LncRNA HOTAIR in ACAS patients and its potential diagnostic significance. **A** qRT-PCR was performed to detect differences in serum HOTAIR levels in ACAS patients and controls. **B** ROC curves based on the levels of HOTAIR drawn in ACAS patients and controls were examined for its diagnostic accuracy. *** $P < 0.001$, compared with Controls

Table 2 Relationship between degree of carotid artery stenosis and indexes

Variables	Univariate			Multivariable		
	OR	95% CI	*P* value	OR	95% CI	*P* value
LncRNA HOTAIR	**0.322**	**0.134–0.771**	**0.011**	**0.289**	**0.107–0.786**	**0.015**
Gender (male/female)	0.899	0.383–**2.064**	0.785			
Age (years)	0.838	0.362–**1.939**	0.680			
BMI (kg/m^2)	**2.517**	**1.065–5.949**	**0.035**	2.609	0.979 –6.953	0.055
FBG (mg/dL)	0.828	0.358–**1.915**	0.659			
TC (mg/dL)	**0.419**	**0.176–0.994**	**0.048**	0.376	0.140 –1.011	0.053
TG (mg/dL)	0.924	0.399–**2.137**	0.853			
HDL (mg/dL)	**0.375**	**0.157–0.898**	**0.028**	0.446	0.168 –1.184	0.105
LDL (mg/dL)	0.760	0.328–**1.760**	0.522			
SBP (mm Hg)	**2.517**	**1.065–5.949**	**0.035**	2.784	1.039 –7.462	0.042
DBP (mm Hg)	1.417	0.611–**3.291**	0.417			
CRP (mg/l)	0.828	0.0358–**1.915**	0.659			

BMI body mass index, *FBG* fasting blood glucose, *TC* total cholesterol, *TG* triglycerides, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *CRP* C-reactive protein

3.5 Serum LncRNA HOTAIR Suppression Predicts the Incidence of CIE

To explore the potential value of HOTAIR in ACAS, the present research also documented the incidence of CIE by follow-up, in which a total of 25 patients (16 TIA, 7 IS, and 2 deaths) developed CIE. In addition, serum HOTAIR was considerably lower in CIE-positive patients compared with CIE-negative ACAS patients ($P<0.01$, Fig. 2A). Based on the mean levels of HOTAIR, ACAS patients were divided into a high HOTAIR group ($n=43$) and a low HOTAIR group ($n=45$). K-M curves confirmed that patients with low HOTAIR were more susceptible to CIE (log-rank $P=0.001$, Fig. 2B). Finally, as shown in Table 3, it was found that in univariate COX analysis, HOTAIR, FBG, LDL, DBP, CRP, and Degree of carotid stenosis were correlated with the development of CIE ($P<0.05$), but in multivariable COX analysis, only serum HOTAIR (HR = 0.244, 95% CI = 0.072–0.824, $P=0.023$) and degree of carotid stenosis (HR = 4.566, 95% CI = 1.206–17.292, $P=0.025$) were risk factors for the development of CIE.

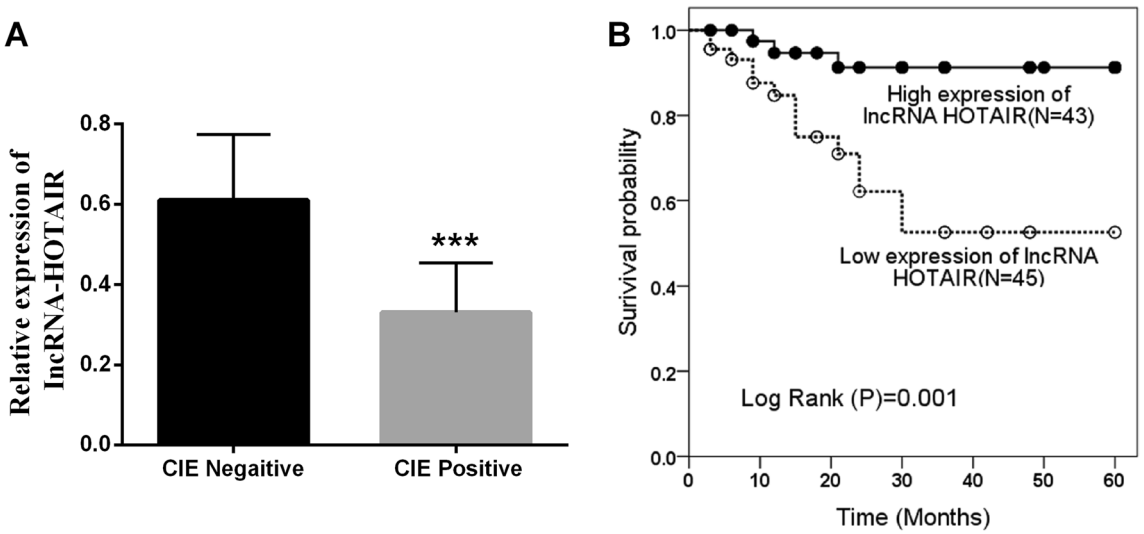

Fig. 2 Relationship between HOTAIR levels and the incidence of CIE in ACAS patients. **A** qRT-PCR was employed to identify serum levels of HOTAIR in CIE-positive and negative patients. **B** K-M survival curves were plotted based on the levels of HOTAIR in ACAS patients to evaluate the occurrence of CIE in patients with different levels of HOTAIR. *** $P<0.001$, compared with CIE Negative group

Table 3 Cox analysis of the independent factors affecting CIEs

Name of index	Univariate			Multivariable		
	HR	95% CI	*P* value	HR	95% CI	*P* value
LncRNA HOTAIR	**0.200**	**0.065–0.612**	**0.005**	**0.244**	**0.072–0.824**	**0.023**
Gender	0.997	0.384–2.584	0.994			
Age	0.850	0.323–2.238	0.742			
BMI	1.039	0.401–2.697	0.937			
FBG	**3.177**	**1.034–9.762**	**0.044**	1.842	0.506–**6.713**	0.354
TC	1.316	0.486–3.565	0.589			
TG	0579	0.219–1.515	0.264			
HDL	0.743	0.285–1.940	0.545			
LDL	**0.344**	**0.121–0.981**	**0.046**	0.827	0.223–3.061	0.776
SBP	1.697	0.627–4.594	0.298			
DBP	**3.365**	**1.097–10.323**	**0.034**	2.208	0.566–8.610	0.254
CRP	**3.624**	**1.181–11.120**	**0.024**	1.333	0.336–5.287	0.683
Degree of carotid stenosis	**6.043**	**1.731–21.097**	**0.005**	**4.566**	**1.206–17.292**	**0.025**

Instructions: *CIE* cerebral ischemic events, *BMI* body mass index, *FBG* fasting blood glucose, *TC* total cholesterol, *TG* triglycerides, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *CRP* C-reactive protein

4 Discussion

CAS as one of the main risk factors for the incidence of CIE is commonly ignored because some patients do not develop symptoms such as TIA and IS, but 14% of IS comes from CAS according to the Oxford Vascular study [28]. Therefore, early diagnosis of ACAS is a primary requirement for determining treatment options and patient care and management. Currently, Doppler ultrasound, computed tomographic angiography (CTA), magnetic resonance angiography (MRA), and digital subtraction angiography image (DSA) are performed for examination of ACAS [29]. Doppler ultrasound is currently the preferred noninvasive screening modality for ACAS due to its lower cost, convenience, and reproducibility. However, its results are susceptible to the operator's skill levels [6]. MRA is limited to long exam times, and stenosis amplification, and is not suitable for patients with metal stents or pacemakers [7]; DAS is invasive, and the contrast agents and X-rays of CTA are potentially dangerous [30]. Meanwhile, these screening methods can only detect already formed atherosclerotic lesions, while dysregulation of molecules such as LncRNA and miRNA from body fluids (serum, saliva) are involved in the physiopathological process of the disease and can not only significantly identify lesions, but also accurately predict disease progression and prognosis. Additionally, they have also received widespread attention for their potential as biomarkers due to their stability, non-invasiveness and easy accessibility.

Growing evidence demonstrates that dysregulation of LncRNAs is participating in the advancement of cardiovascular disease and has targets for development into therapeutic strategies [31]. Such as LncRNA H19 [32], LncRNA SNHG1 [33], and LncRNA CDKN2B-AS1 [34] have been indicated to be involved in the progression of CAS. As one of the numerous LncRNAs, HOTAIR has been described as a diagnostic marker for type 2 diabetes and predicts its chronic complications [35]. HOTAIR was markedly downregulated in rats with myocardial ischemia–reperfusion injury [36] and promoted myocardial inflammation and apoptosis [37]. The polymorphism of HOTAIR serves as a potential diagnostic marker for coronary artery disease [18]. In addition, dysregulation of HOTAIR is involved in cerebral ischemia–reperfusion injury [38] and hypoxic-ischemic encephalopathy [39]. Atherosclerosis is the pathological basis for the development of CAS, and HOTAIR was identified to be significantly lower in AS patients [20]. Although HOTAIR has been extensively reported in other diseases, however, its role in ACAS is unclear. In our research, it was confirmed that serum HOTAIR levels were statistically different in ACAS patients and healthy individuals and were considerably lower compared to controls. The results are consistent with the levels in patients with atherosclerosis in previous studies and remind us that dysregulation of HOTAIR may participate in the progression of ACAS.

To further explore the clinical value of HOTAIR in patients with ACAS, we performed logistic regression of patients' clinical data and serum HOTAIR levels to identify factors influencing the degree of carotid stenosis in patients. The results confirmed that serum HOTAIR levels correlated with the degree of severe carotid artery stenosis. Since the degree of carotid stenosis serves as an important indicator for the diagnosis of CAS and

its treatment, it is also an appreciable risk factor for the development of CIE [40]. Therefore, dysregulated HOTAIR has potential connections with the diagnosis of ACAS and the incidence of CIE.

In our study, We performed internal validation based on previous studies [41, 42] and reported the predictive performance in terms of scaled Brier score with empirical 95% confidence intervals, and found overall good performance. Serum HOTAIR was found to be a potential diagnostic biomarker that can identify ACAS patients from controls with high sensitivity and specificity. ACAS as a leading factor for future CIE, we followed the patients for 5 years and found that the degree of carotid stenosis and HOTAIR level were independent risk factors for the development of CIE. More importantly, we found that CIE-positive patients had lower levels of HOTAIR than CIE-negative patients. K-M curves also confirmed that patients with low levels of HOTAIR were more susceptible to CIE than those with high levels of HOTAIR. Clinical risk and prediction can contribute to clinical and cost-effective targeted screening programs. In our study, serum HOTAIR significantly predicted the risk of CIR in individuals with ACAS who were at high risk of developing CIE.

5 Limitations

This study does have several limitations that need to be considered when interpreting the findings.

Firstly, the small size of the sample with the limited number of events, the lack of any independent validation sample, the loss of follow-up, and the potential for missing (or misdiagnosis of) future cerebral ischemic events are all limitations of this preliminary study. Additionally, this study was limited by the fact that traditional risk factors such as smoking and alcohol consumption were not included on time, and we will expand the sample size for a multicenter study in further studies to include these risk factors while further investigating the potential mechanisms underlying the role of HOTAIR in ACAS. Furthermore, invasive blood sampling is required to determine the level of HOTAIR, which also represents a potential limitation of HOTAIR clinical practice. This will be addressed in our follow-up study.

6 Conclusions

The principal findings of the current study are that HOTAIR was markedly reduced in patients with ACAS. It's the levels that can identify ACAS patients and predict the incidence of CIE. In conclusion, we demonstrated for the first time that HOTAIR may be a feasible biomarker for ACAS.

Abbreviations

ACAS	Asymptomatic carotid artery stenosis
AMI	Acute myocardial infarction
AS	Atherosclerosis
CAS	Carotid artery stenosis
CI	Confidence interval
CIE	Cerebral ischemic events
CT	Computed tomography
CTA	Computed tomographic angiography
DSA	Digital subtraction angiography image
HOTAIR	Hox transcript antisense intergenic RNA
IS	Ischemic stroke
K-M	Kaplan–Meier
LncRNAs	Long non-coding RNAs
MI	Myocardial infarction
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
NASCET	North American symptomatic carotid endarterectomy trial
qRT-PCR	Quantitative real-time polymerase chain reaction
ROC	Receiver operating characteristic
TIA	Transient ischemic attack

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s44200-024-00051-x.

Below is the link to the electronic supplementary material.Supplementary file1 (DOCX 605 KB)

Supplementary file2 (DOCX 91 KB)

Supplementary file3 (DOCX 15 KB)

Acknowledgements
Not applicable.

Author Contributions
LL and YL made substantial contributions to the conception and design, acquisition of data, analysis and interpretation of data, and the draft of the manuscript. PCF, CGT and LL revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Funding
No funding was received for conducting this study.

Data Availability
The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of Interest
The authors report there are no competing interests to declare.

Ethics Approval and Consent to participate
All procedures were approved by the ethics committee of the Affiliated Hospital of Panzhihua University, and all the research subjects provided the informed consent form.

Consent for Publication
Not applicable.

Author details
[1]Department of Neurology, China Resources & WISCO General Hospital, Wuhan 430080, China. [2]Department of Neurology, Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing 100091, China. [3]Department of General Practice, Affiliated Hospital of Panzhihua University, 27 Taoyuan Street, Panzhihua 617200, Sichuan, China.

Received: 26 November 2023 Accepted: 25 June 2024
Published: 4 July 2024

References

1. Badacz R, Przewlocki T, Gacon J, Stepien E, Enguita FJ, Karch I, et al. Circulating miRNA levels differ with respect to carotid plaque characteristics and symptom occurrence in patients with carotid artery stenosis and provide information on future cardiovascular events. Postepy Kardiol Interwencyjnej. 2018;14:75–84.
2. Yoshida K, Miyamoto S, Group S-KS. Stratification by Multidimensional Approach for Rational Treatment of Asymptomatic Carotid Stenosis (SMART-K Study): Study Protocol. Neurol Med Chir (Tokyo). 2020;60:10–6.
3. Luo T, Cui S, Bian C, Yu X. Crosstalk between TGF-beta/Smad3 and BMP/BMPR2 signaling pathways via miR-17-92 cluster in carotid artery restenosis. Mol Cell Biochem. 2014;389:169–76.
4. Pewowaruk RJ. Simple models of complex mechanics for improved hypertension care: learning to de-stiffen arteries. Artery Res. 2023;29:94–100.
5. Wang W, Wu J, Liu P, Tang X, Pang H, Xie T, et al. Urinary proteomics identifying novel biomarkers for the diagnosis and phenotyping of carotid artery stenosis. Front Mol Biosci. 2021;8: 714706.
6. Tan EJ, Zhang S, Tirukonda P, Chong LR. REACT - A novel flow-independent non-gated non-contrast MR angiography technique using magnetization-prepared 3D non-balanced dual-echo dixon method: Preliminary clinical experience. Eur J Radiol Open. 2020;7: 100238.
7. Shrivastava A, Srivastava T, Saxena R. CT Angiographic evaluation of pattern and distribution of stenosis and its association with risk factors among indian ischemic stroke patients. Pol J Radiol. 2016;81:357–62.
8. Zhu Z, Hu W, Chen R, Xiong R, Wang W, Shang X, et al. Retinal age gap as a predictive biomarker of stroke risk. BMC Med. 2022;20:466.
9. Zhang Q, Huang XM, Liao JX, Dong YK, Zhu JL, He CC, et al. LncRNA HOTAIR Promotes Neuronal Damage Through Facilitating NLRP3 Mediated-Pyroptosis Activation in Parkinson's Disease via Regulation of miR-326/ELAVL1 Axis. Cell Mol Neurobiol. 2021;41:1773–86.
10. Xin X, Li Q, Fang J, Zhao T. LncRNA HOTAIR: a potential prognostic factor and therapeutic target in human cancers. Front Oncol. 2021;11: 679244.
11. Zhang D, Wang B, Ma M, Yu K, Zhang Q, Zhang X. lncRNA HOTAIR protects myocardial infarction rat by sponging miR-519d-3p. J Cardiovasc Transl Res. 2019;12:171–83.
12. Huang Y, Wang Y, Liu X, Ouyang Y. Silencing lncRNA HOTAIR improves the recovery of neurological function in ischemic stroke via the miR-148a-3p/KLF6 axis. Brain Res Bull. 2021;176:43–53.
13. Lang Y, Li Y, Yu H, Lin L, Chen X, Wang S, et al. HOTAIR drives autophagy in midbrain dopaminergic neurons in the substantia nigra compacta in a mouse model of Parkinson's disease by elevating NPTX2 via miR-221-3p binding. Aging (Albany NY). 2020;12:7660–78.
14. Chen J, Li X, Zhao F, Hu Y. HOTAIR/miR-17-5p Axis is Involved in the Propofol-Mediated Cardioprotection Against Ischemia/Reperfusion Injury. Clin Interv Aging. 2021;16:621–32.
15. Lai Y, He S, Ma L, Lin H, Ren B, Ma J, et al. HOTAIR functions as a competing endogenous RNA to regulate PTEN expression by inhibiting miR-19 in cardiac hypertrophy. Mol Cell Biochem. 2017;432:179–87.
16. Greco S, Zaccagnini G, Perfetti A, Fuschi P, Valaperta R, Voellenkle C, et al. Long noncoding RNA dysregulation in ischemic heart failure. J Transl Med. 2016;14:183.
17. Gao L, Liu Y, Guo S, Yao R, Wu L, Xiao L, et al. Circulating long noncoding RNA HOTAIR is an essential mediator of acute myocardial infarction. Cell Physiol Biochem. 2017;44:1497–508.
18. Kim IJ, Lee JY, Park HW, Park HS, Ko EJ, Sung JH, et al. Association between HOTAIR lncRNA Polymorphisms and Coronary Artery Disease Susceptibility. J Pers Med. 2021;11.
19. Peng Y, Meng K, Jiang L, Zhong Y, Yang Y, Lan Y, et al. Thymic stromal lymphopoietin-induced HOTAIR activation promotes endothelial cell proliferation and migration in atherosclerosis. Biosci Rep. 2017;37.
20. Pang JL, Wang JW, Hu PY, Jiang JS, Yu C. HOTAIR alleviates ox-LDL-induced inflammatory response in Raw264.7 cells via inhibiting NF-kappaB pathway. Eur Rev Med Pharmacol Sci. 2018;22:6991–8.
21. Yi L, Tang J, Shi C, Zhang T, Li J, Guo F, et al. Pentraxin 3, TNF-alpha, and LDL-C are associated with carotid artery stenosis in patients with ischemic stroke. Front Neurol. 2019;10:1365.
22. Munir I, Mehmood T, Islam K, Soni L, McFarlane SI. Thyrotoxic periodic paralysis with sensory deficits in young african american male: a case report and literature review. Am J Med Case Rep. 2019;7:138–42.
23. Olindo S, Chausson N, Signate A, Mecharles S, Hennequin JL, Saint-Vil M, et al. Stroke recurrence in first-ever symptomatic carotid web: a cohort study. J Stroke. 2021;23:253–62.
24. Wu H, Liu A. Long non-coding RNA NEAT1 regulates ferroptosis sensitivity in non-small-cell lung cancer. J Int Med Res. 2021;49:300060521996183.
25. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. Ann Intern Med. 2015;162:55–63.
26. Endo H, Yoshida K, Kurosaki Y, Sadamasa N, Narumi O, Chin M, et al. [Detection of intraplaque hemorrhage with use of screening black-blood MRI]. No Shinkei Geka. 2009;37:249–53.
27. Si W, He P, Wang Y, Fu Y, Li X, Lin X, et al. Complement complex C5b–9 levels are associated with the clinical outcomes of acute ischemic stroke and carotid plaque stability. Transl Stroke Res. 2019;10:279–86.
28. Badacz R, Kablak-Ziembicka A, Urbanczyk-Zawadzka M, Banys RP, Musialek P, Odrowaz-Pieniazek P, et al. Magnetic resonance imaging and clinical outcome in patients with symptomatic carotid artery stenosis after carotid artery revascularization. Postepy Kardiol Interwencyjnej. 2017;13:225–32.
29. Hou J, Gao MY, Pan AZ, Wang QD, Liu B, Jin YB, et al. Relationship between circle of willis variations and cerebral or cervical arteries stenosis investigated by computer tomography angiography and multitask convolutional neural network. J Healthc Eng. 2021;2021:6024352.
30. Liu Y, Liu G, Yang D, Xu B. Application of Linear Gradient Magnetic Field in Arterial Profile Scanning Imaging. Sensors (Basel). 2020;20.
31. Liu K, Zhang L, Li X, Zhao J. High expression of lncRNA HSD11B1-AS1 indicates favorable prognosis and is associated with immune infiltration in cutaneous melanoma. Oncol Lett. 2022;23:54.
32. Zhou F, Hu X, Feng W, Li M, Yu B, Fu C, et al. LncRNA H19 abrogates the protective effects of curcumin on rat carotid balloon injury via activating Wnt/beta-catenin signaling pathway. Eur J Pharmacol. 2021;910: 174485.
33. Ma H, Dong A. Dysregulation of lncRNA SNHG1/miR-145 axis affects the biological function of human carotid artery smooth muscle cells as a mechanism of carotid artery restenosis. Exp Ther Med. 2021;21:423.
34. Ma H, Dong A. Long non-coding RNA cyclin-dependent kinase inhibitor 2B antisense ribonucleic acid 1 is associated with in-stent restenosis and promotes human carotid artery smooth muscle cell proliferation and migration by sponging miR-143-3p. Exp Ther Med. 2021;21:234.
35. Wang H, Xia Y, Zhang Y. Diagnostic significance of serum lncRNA HOTAIR and its predictive value for the development of chronic complications in patients with type 2 diabetes mellitus. Diabetol Metab Syndr. 2021;13:97.
36. Fang J, Zheng W, Hu P, Wu J. Investigating the effect of lncRNA HOTAIR on apoptosis induced by myocardial ischemia-reperfusion injury. Mol Med Rep. 2021;23.
37. Ni SY, Xu WT, Liao GY, Wang YL, Li J. LncRNA HOTAIR Promotes LPS-Induced Inflammation and Apoptosis of Cardiomyocytes via Lin28-Mediated PDCD4 Stability. Inflammation. 2021;44:1452–63.
38. Ma NH, Zhang MH, Yang JX, Sun ZJ, Yuan F, Qiu XL. Long noncoding RNA HOTAIR sponging miR-211 regulates cerebral ischemia-reperfusion injury. J Biol Regul Homeost Agents. 2020;34:2209–14.
39. Wang Y, Mao J, Li X, Wang B, Zhou X. lncRNA HOTAIR mediates OGD/R-induced cell injury and angiogenesis in a EZH2-dependent manner. Exp Ther Med. 2022;23:99.
40. Chao CH, Wu CL, Huang WY. Association between estimated glomerular filtration rate and clinical outcomes in ischemic stroke patients with high-grade carotid artery stenosis. BMC Neurol. 2021;21:124.
41. Gatto F, Dabestani S, Bratulic S, Limeta A, Maccari F, Galeotti F, et al. Plasma and urine free glycosaminoglycans as monitoring biomarkers in nonmetastatic renal cell carcinoma-a prospective cohort study. Eur Urol Open Sci. 2022;42:30–9.
42. Li L, Wang L, Lu L, Zhu T. Machine learning prediction of postoperative unplanned 30-day hospital readmission in older adult. Front Mol Biosci. 2022;9: 910688.