Role of circulating long non-coding RNA for the improvement of the predictive ability of the CHA_2DS_2–VASc score in patients with atrial fibrillation

Yuanbo Zhang[1], Duan Wang[2], Na Wu[1], Xinghua Chen[3], Zhiquan Yuan[1,2], Xiaoyue Jia[1], Chengying Li[1], Qin Hu[1], Yanxiu Chen[3], Zhihui Zhang[3], Li Zhong[2], Yafei Li[1]

[1]Department of Epidemiology, College of Preventive Medicine, Army Medical University (Third Military Medical University), Chongqing 400038, China;
[2]Department of Cardiology, Third Affiliated Hospital of Chongqing Medical University, Chongqing 401120, China;
[3]Department of Cardiology, Southwest Hospital, Army Medical University (Third Military Medical University), Chongqing 400038, China.

Abstract

Background: The CHA_2DS_2–VASc score was initially applied to stratify stroke risk in patients with atrial fibrillation (AF) and was found to be effective in predicting all-cause mortality outcomes. To date, it is still unclear whether circulating long non-coding RNAs (lncRNAs) as emerging biomarkers, can improve the predictive power of the CHA_2DS_2–VASc score in stroke and all-cause mortality.

Methods: Candidate lncRNAs were screened by searching the literature and analyzing previous RNA sequencing results. After preliminary verification in 29 patients with AF, the final selected lncRNAs were evaluated by Cox proportional hazards regression in 192 patients to determine whether their relative expression levels were associated with stroke and all-cause mortality. The c-statistic, net reclassification improvement (NRI), and integrated discrimination improvement of the patients were calculated to evaluate the discrimination and reclassification power for stroke and all-cause mortality when adding lncRNA expression levels to the CHA_2DS_2–VASc score model.

Results: Five plasma lncRNAs associated with stroke and all-cause mortality in AF patients were selected in our screening process. Patients with elevated H19 levels were found to have a higher risk of stroke (hazard ratio [HR] 3.264, 95% confidence interval [CI]: 1.364–7.813, $P = 0.008$). Adding the H19 expression level to the CHA_2DS_2–VASc score significantly improved the discrimination and reclassification power of the CHA_2DS_2–VASc score for stroke in AF patients. In addition, the H19 level showed a marginally significant association with all-cause mortality (HR 2.263, 95% CI: 0.889–5.760, $P = 0.087$), although it appeared to have no significant improvement for the CHA_2DS_2–VASc model for predicting all-cause mortality.

Conclusions: Plasma expression of H19 was associated with stroke risk in AF patients and improved the discriminatory power of the CHA_2DS_2–VASc score. Therefore, lncRNA H19 served as an emerging non-invasive biomarker for stroke risk prediction in patients with AF.

Keywords: Atrial fibrillation; Long non-coding RNA; H19; Prognosis

Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia in adults around the world.[1] Studies have demonstrated that it is associated with an increased risk of stroke and all-cause mortality, placing a serious burden on patients and society.[2,3] Furthermore, as populations age, the prevalence and incidence of AF will increase.[4] To optimize the therapy and management of AF, it is crucial to correctly stratify patients according to their prognosis.[5] The CHA_2DS_2–VASc score has been the most common and convenient tool for stroke risk assessment in patients with AF. Meanwhile, its predictive power in terms of all-cause mortality is increasingly being recognized.[6-8] Potential circulating disease biomarkers are gradually being identified and used in clinical practice, and a biomarker-based risk score has recently been successfully developed to predict stroke and death in patients with AF recently.[9]

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Correspondence to: Yafei Li, Department of Epidemiology, College of Preventive Medicine, Army Medical University (Third Military Medical University), No. 30 Gaotanyan Street, Chongqing 400038, China
E-Mail: liyafei2008@tmmu.edu.cn, liyafei2008@hotmail.com
Li Zhong, Cardiovascular Disease Center, Third Affiliated Hospital of Chongqing Medical University, Chongqing 401120, China
E-Mail: zhongli28@hotmail.com

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Genetic and epigenetic factors are believed to be important in AF.[10] During the past decade, non-coding RNAs (ncRNAs), particularly microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have been increasingly recognized as key regulators and potential biomarkers in numerous diseases. Accumulative studies have revealed that some miRNAs significantly modulate the occurrence and development of AF.[11-13] MiRNAs in plasma/serum can be used as a potential non-invasive biomarkers of many cardiac pathologies, including AF.[14-16] LncRNAs have received much more attention in recent years since their aberrant expression is thought to be associated with cardiovascular disease risks.[17] In particular, due to their stability in circulating peripheral blood, lncRNAs may also serve as non-invasive biomarkers and guide clinical decisions by facilitating diagnosis, prognosis, and disease classification. However, research exploring the potential role of lncRNAs in AF is still limited and their impact on the prognosis is still largely unclear.

Previously, we have identified the expression profiles of lncRNAs in patients with AF by RNA sequencing.[18] In this study, we selected a list of stable lncRNAs in plasma based on our previous findings and published studies in the database. We investigated their prognostic values in a cohort of AF patients to determine whether lncRNAs could improve the risk stratification ability of the CHA_2DS_2–VASc score.

Methods

Ethical approval

Approval was obtained from the Ethics Committee of Army Medical University (Approval No. KY2020231). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 *Helsinki declaration* and its later amendments or comparable ethical standards. Written informed consent was obtained from each participant included in the study.

Screening strategy for lncRNAs

As described in detail in our previous study, we collected the atrial tissues of seven AF patients which were then paired with matched controls for Hiseq/Proton (Thermo Fisher Scientific, Waltham, MA, USA) RNA sequencing to examine the expression profiles of lncRNAs in AF.[18] Some of these differentially expressed lncRNAs were then validated by reverse transcription-quantitative real-time PCR (qRT-PCR) in 35 pairs of AF patients and control atrial tissue samples. Based on the sequencing results, lncRNAs were selected with filter criteria as follows: (1) expression fold change >1.5 ($P < 0.05$ and false discovery rate < 0.05); (2) the reads in each sample >2; (3) the length of lncRNA within the range of 500 to 3000 nt; and (4) excluding overlap with exons of other genes. To avoid missing some lncRNAs with fold change between 1.5 and 2.0 that could also predict the prognosis of AF, we set up an expression fold change >1.5.

Literature search

We performed a systematic literature search to screen lncRNAs that have been studied in cardiovascular disease and expressed in plasma. We identified relevant published studies in PubMed and Embase by using the following terms: (a) "Long non-coding RNA" or LncRNA and (b) "cardiovascular disease" and (c) plasma. We manually searched the reference list of selected articles to ensure that all relevant papers had been identified. We identified 67 and 24 relevant papers in the PubMed and Embase databases, respectively. After critical review, we excluded duplicates, reviews, letters, and studies that were not in the field of interest and finally included 24 studies that reported 16 lncRNAs: H19, LIPCAR, UCA1, GAS5, CoroMarker, NRON, MHRT, IFNG-AS1, ANRIL, CHROME, DKFZP434I0714, MIAT, AK098656, HOTAIR, RMST, and BACE1-AS. Second, to further narrow down the list of the 16 lncRNAs, we searched through literature that focused on studies of the mechanism of lncRNAs in cardiomyocytes or cardiac fibroblasts in PubMed by using the following terms: (a) H19 or LIPCAR or UCA1 or GAS5 or CoroMarker or NRON or MHRT or IFNG-AS1 or ANRIL or CHROME or DKFZP434I0714 or MIAT or AK098656 or HOTAIR or RMST or BACE1-AS and (b) "cardiac fibroblast" or "cardiomyocyte" and (c) "cardiovascular disease". We identified 37 papers in the PubMed database, and a total of seven lncRNAs were included (including H19, UCA1, GAS5, MHRT, MIAT, HOTAIR, and BACE1-AS). Finally, we selected lncRNA H19, UCA1, and GAS5 for further research because these three lncRNAs were stably expressed in plasma according to our preliminary experiment. The literature screening process is demonstrated in the flow chart in Supplementary Figure 1, http://links.lww.com/CM9/B117.

Patient cohort and data collection

We continuously recruited AF patients treated in a hospital from southwest China, who were diagnosed according to the 2010 ESC Guideline between December 2013 and August 2015.[1] Patients were excluded from this study if they were diagnosed with structural heart disease, moderate to severe mitral stenosis, malignant tumor, prosthetic valve replacement, sepsis, hyperthyroidism, history of drug abuse, and undergoing ablation. We combined medical records with standardized subject interviews to obtain clinical and demographic data for each participant in the cohort. According to the CHA_2DS_2–VASc score, patients who had congestive heart failure, had hypertension, were 65 to 74 years old, had diabetes, had vascular disease, and were female were given a score of 1, and patients who were ≥75 years old and had a history of stroke, transient ischemic attack (TIA), or thromboembolism were given a score of 2.

According to the extended formula of Cox regression sample size estimation by Hsieh and Lavori[19], the sample size was estimated by PASS11 software (NCSS LLC, Kaysville, UT, USA), suggesting that 173 subjects were needed. Considering 10% loss of follow-up rate, at least 190 patients were needed.

Follow-up and study outcomes

The primary outcomes for the current analysis were stroke and all-cause mortality. Stroke was defined as a hemorrhagic or ischemic event with associated clinical features after discharge. The diagnosis of stroke was confirmed by tracking medical records. All the subjects in the study were followed closely and a routine telephone interview was conducted every year. Their vital statuses and causes of death were ascertained annually through electronic medical records, information obtained from next-to-skin or family members, and from death certificates.

Extraction of total RNA

The peripheral blood samples of all subjects were drawn into a test tube containing EDTA and processed within 1 h after collection. Subsequently, blood was centrifuged at 2000 × *g* for 20 min at 4°C to obtain plasma. The isolated supernatants (plasma fractions) were aliquoted into cryotubes and stored at −80°C until assayed. According to the manufacturer's protocol, we applied a miRNeasy Serum/Plasma Kit (Qiagen, Redwood City, CA, USA) to extract the total RNA of plasma. All steps of RNA extraction used RNase-free materials. The extracted RNA samples were stored at −80°C.

cDNA synthesis and qRT-PCR analysis

We applied the PrimeScript™ fragments RT reagent Kit with gDNA Eraser (TaKaRa-Bio, Otsu, Japan) to reverse transcribe the isolated total RNA into cDNA. A S1000™ Thermal Cycler (BIO-RAD, CA, USA) was used for reverse transcription and the final cDNA samples were stored at −20°C.

qRT-PCR was performed using the TB Green™ Premix Ex Taq™ II (TaKaRa-Bio), according to the manufacturer's protocol. Patient plasma was randomly mixed as an interplate control. The results were normalized to the expression levels of human β-actin. The ΔΔCt was used to show the gene expression levels. $\Delta\Delta Ct = (Ct_{target} - Ct_{\beta\text{-}actin})_{sample} - (Ct_{target} - Ct_{\beta\text{-}actin})_{control}$. The fold enrichment was determined as $2^{-\Delta\Delta Ct}$.

Statistical analysis

For continuous variables, normality was assessed using a Shapiro–Wilk normality test. Gaussian distributed data are presented as the mean ± standard deviation, and non-Gaussian distributed variables are presented as the median with interquartile range (IQR). A *t* test or Mann–Whitney *U* test was used to examine group differences. Categorical data are presented as counts and percentiles. X-tile software 3.6.1 (Yale University, New Haven, CT, USA) was used to calculate the cutoff values of the normalized expression of ncRNAs.[20] X-tile also provide a Monte Carlo *P* value to evaluate multiple cutoff points and find the best value according to the best *P* value.

Univariate Cox proportion hazard models were applied to evaluate the relationship between clinical variables and efficacy outcomes, and clinical variables with $P < 0.10$ were included in multivariable Cox regression analysis with a backward method to determine ncRNA prognostic values. Survival analysis was performed using the Kaplan–Meier method and log-rank test for survival curves. Sensitivity analysis was performed to identify confounding factors using a univariate Cox model with $P < 0.05$.

The performance of the model was evaluated in terms of discrimination, reclassification, and calibration abilities. A c-statistic was used to test and compare the discriminatory performance of the Cox regression model to evaluate the prognostic accuracy by adding the relative expression levels of lncRNAs to the CHA_2DS_2–VASc score.[21] The receiver operator characteristic (ROC) curves of the CHA_2DS_2–VASc score and CHA_2DS_2–VASc score combined with H19 were compared. Additionally, for reclassification, improvement in predictive accuracy was evaluated by calculating the integrated discrimination index (IDI) and net reclassification improvement (NRI). The Hosmer–Lemeshow goodness-of-fit test (HLS) and calibration curve were applied to evaluate the calibration performance of the models. As described by Vickers and Elkin[22] to assess the net benefit and clinical usefulness of the lncRNA and CHA_2DS_2–VASc combined score in comparison to the original CHA_2DS_2–VASc score, a decision curve analysis (DCA) was also performed. Statistical analyses were performed using SPSS version 23.0 (SPSS, Chicago, IL, USA) and R 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). Two-tailed *P* values < 0.05 were considered to be statistically significant.

Results

LncRNA screening

According to the filter criteria, 11 lncRNAs (five upregulated and six downregulated) were initially selected from the sequencing results, and three lncRNAs (H19, UCA1, and GAS5) were selected from published studies [Figure 1]. All candidate lncRNAs are listed in Supplementary Tables 1 and 2, http://links.lww.com/CM9/B117. The sequencing screening flow chart is listed in Supplementary Figure 2, http://links.lww.com/CM9/B117.

Then, the candidate lncRNAs were preliminarily verified by qRT-PCR in plasma from 29 patients to ensure that the selected lncRNAs had a stable expression in plasma. The PCR primer sequences are shown in Supplementary Table 3, http://links.lww.com/CM9/B117. Finally, we short listed five lncRNAs (NDUFV2P1, RPL18AP3, H19, UCA1, and GAS5) with stable expression in plasma for subsequent cohort study.

Baseline characteristics of the patient cohort

A total of 192 AF patients (99 male, median age was 68 years) were included in our study and 15 were lost to follow-up. Supplementary Table 4, http://links.lww.com/CM9/B117 shows the demographic and clinical characteristics of the included subjects. In all included patients, the median CHA_2DS_2–VASc score was 3 (IQR, 2–4), and 76.6% ($n = 147$) of patients were at high risk

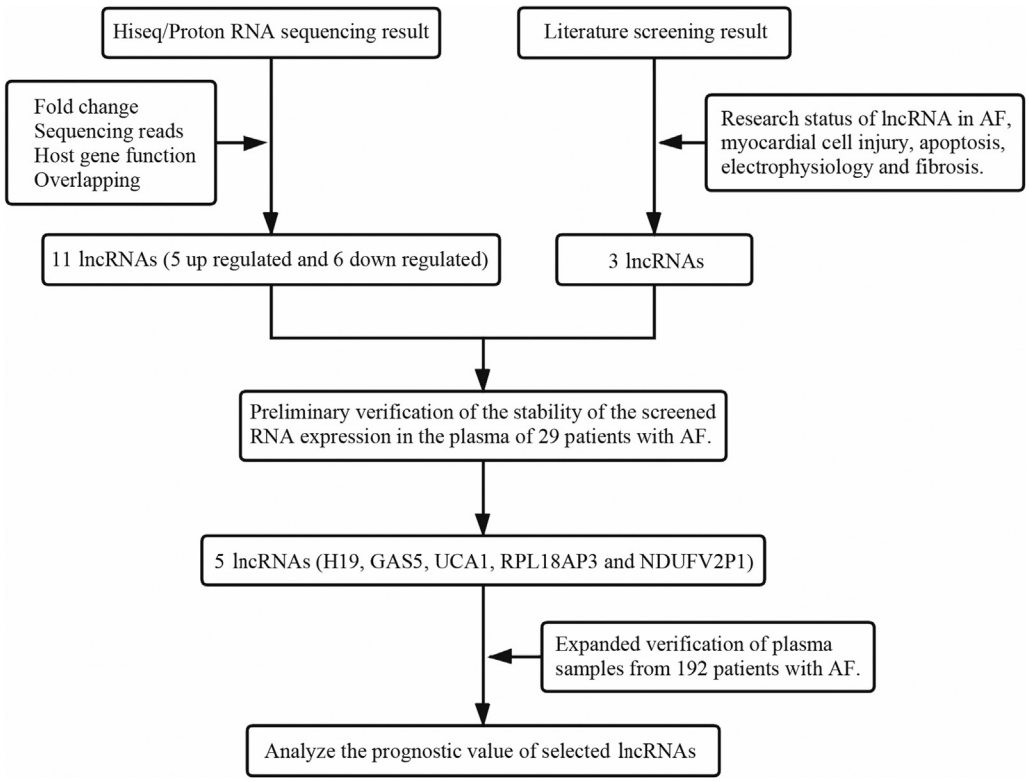


Figure 1: Flow chart of lncRNA screening. AF: Atrial fibrillation; lncRNA: Long noncoding RNA.

(CHA_2DS_2–VASc score ≥2) of stroke. A total of 23 strokes (5.93 per 100 person-years) and 24 deaths (5.76 per 100 person-years) occurred during the median follow-up period of 26 months. The cutoff value of each selected lncRNA for outcomes is presented in Supplementary Table 5, http://links.lww.com/CM9/B117.

Association of plasma lncRNAs with stroke and all-cause mortality in AF

By univariate Cox proportional hazards analysis, we found that the age and medical history of heart failure among clinical variables were related to all-cause mortality, whereas diabetes, TIA or previous stroke, vascular disease, and antiarrhythmic therapies were risk factors for stroke [Supplementary Table 6, http://links.lww.com/CM9/B117]. These clinical variables were adjusted as covariates in the subsequent multivariable analysis. H19 (hazard ratio [HR] 2.636, 95% confidence interval [CI]: 1.159–5.993, $P = 0.021$), GAS5 (HR 3.557, 95% CI:1.364–9.272, $P = 0.009$), and RPL18AP3 (HR 2.834, 95% CI:1.050–7.649, $P = 0.04$) were found to be associated with the risk of stroke [Table 1]. For all-cause mortality, patients with increased H19 showed a higher risk for all-cause mortality (HR 2.333, 95% CI: 0.918–5.930) but with a borderline statistical significance ($P = 0.075$) [Table 1].

In the multivariable analysis, patients with higher H19 levels had an increased risk of stroke after adjusting for covariates (HR 3.264, 95% CI:1.364–7.813, $P = 0.008$). The Kaplan–Meier survival curves also showed that elevated H19 levels were a significant predictor for stroke in a log-rank test ($P = 0.016$) [Figure 2A and Table 1]. Elevated H19 levels showed a marginally significant association with all-cause mortality (HR 2.263, 95% CI: 0.889–5.760, $P = 0.087$) [Figure 2B and Table 1]. We performed sensitivity analyses to identify confounding factors using a univariate Cox model with $P < 0.05$, and the results were not altered (data not shown).

Predictive ability of the CHA_2DS_2–VASc score combined with H19 for stroke and all-cause mortality

For the prediction of stroke, after adding H19 expression to the CHA_2DS_2–VASc score, the c-statistics were significantly increased from 0.707 (95% CI: 0.621–0.792) to 0.744 (95% CI: 0.661–0.828), $P = 0.022$. The addition of H19 to the CHA_2DS_2–VASc score yielded a significantly positive IDI and NRI for the prediction of stroke [Table 2]. The ROC analysis revealed that the AUC of the score with the addition of the H19 relative expression level appeared to be better than that of the CHA_2DS_2–VASc score [Figure 3]. The model exhibited good calibration performance in both stroke (HLS $P = 0.682$) and all-cause mortality (HLS $P = 0.228$) outcomes [Table 2 and Figure 4]. The DCA results indicated that the CHA_2DS_2–VASc score has higher net benefits and clinical applicability after modification [Figure 5]. For the all-cause mortality, the CHA_2DS_2–VASc score combined with H19 levels appeared to increase the model's discrimination [c-statistics from 0.658 (95% CI: 0.568–0.748) to 0.684 (95% CI: 0.595–0.773)], and reclassification ability, but with no statistical significance.

Table 1: Association between ncRNAs and outcomes in the study population.

Variables	*N* at Risk (*N* with events)	Univariable model			Multivariable model		
		Beta coefficients	Adjusted HR (95% CI)	*P* value	Beta coefficients	Adjusted HR (95% CI)	*P* value
Stroke							
H19							
Low	132 (12)	–	Reference	–	–	Reference	–
High	55 (11)	0.969	2.636 (1.159, 5.993)	0.021	1.183	3.264 (1.364, 7.813)	0.008
UCA1							
Low	90 (9)	–	Reference	–	–	Reference	–
High	49 (7)	0.366	1.442 (0.537, 3.873)	0.468	−0.13	0.878 (0.294, 2.624)	0.815
GAS5							
Low	97 (7)	–	Reference	–	–	Reference	–
High	61 (11)	1.269	3.557 (1.364, 9.272)	0.009	0.585	1.794 (0.586, 5.498)	0.306
NDUFV2P1							
Low	84 (6)	–	Reference	–	–	Reference	–
High	106 (17)	0.887	2.429 (0.956, 6.171)	0.062	0.551	1.734 (0.655, 4.592)	0.268
RPL18AP3							
Low	172 (18)	–	Reference	–		Reference	
High	20 (5)	1.042	2.834 (1.050, 7.649)	0.040	0.557	1.746 (0.561, 5.435)	0.336
All-cause mortality							
H19							
Low	159 (17)	–	Reference	–		Reference	–
High	28 (6)	0.847	2.333 (0.918, 5.930)	0.075	0.817	2.263 (0.889, 5.760)	0.087
UCA1							
Low	77 (12)	–	Reference	–	–	Reference	–
High	62 (5)	−0.663	0.516 (0.182, 1.464)	0.213	−0.23	0.794 (0.275, 2.299)	0.671
GAS5							
Low	39 (7)	–	Reference	–	–	Reference	–
High	119 (13)	−0.461	0.631 (0.254, 1.564)	0.320	−0.166	0.847 (0.337, 2.129)	0.724
NDUFV2P1							
Low	35 (6)	–	Reference	–	–	Reference	–
High	155 (17)	−0.427	0.653 (0.257, 1.657)	0.369	−0.121	0.886 (0.346, 2.266)	0.800
RPL18AP3							
Low	175 (21)	–	Reference	–	–	Reference	–
High	17 (3)	0.543	1.722 (0.513, 5.784)	0.379	0.738	2.093 (0.617, 7.096)	0.236

Multivariable Cox proportional hazards model for stroke adjusted for diabetes mellitus, vascular disease, TIA or previous stroke and antiarrhythmic therapy, and all-cause mortality adjusted for age and heart failure. CI: Confidence interval; HR: Hazard ratio; ncRNAs: Non-coding RNAs; TIA: Transient ischemic attack.

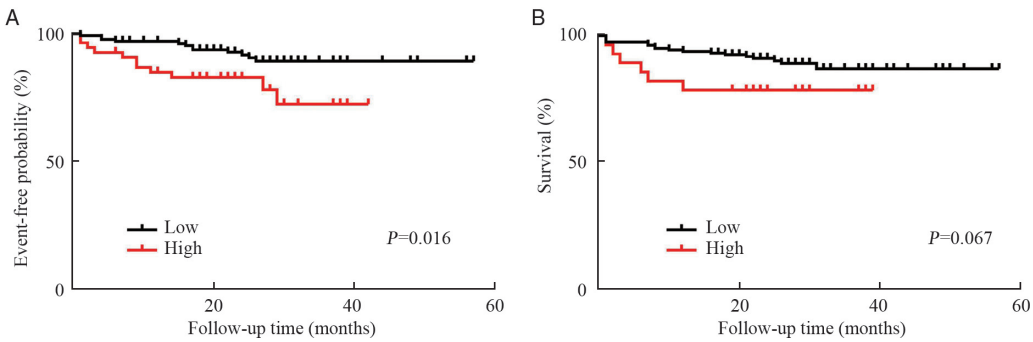
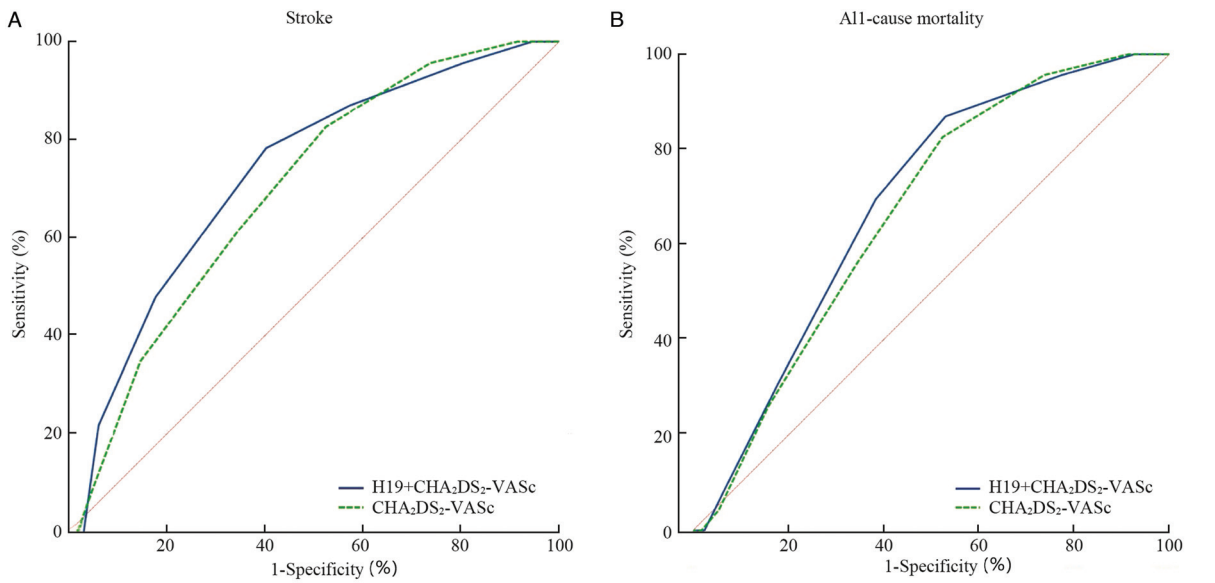


Figure 2: Kaplan–Meier event-free survival curves for relative expression of lncRNA in plasma. There is a significant association between the relative expression of H19 and survival rates for stroke. (A) High lncRNA H19 expression was associated with a slight, but not significant, shortened survival. (B) High expression of lncRNA H19 was associated with significantly poor prognostic outcome for stroke. lncRNAs: Long noncoding RNAs.

Table 2: Discrimination and reclassification for all-cause mortality and stroke.

Models	Discrimination				Reclassification				
	C-statistic (95% CI)	*P* value	Improvement in C-statistic (95% CI)	*P* value	NRI (%) (95% CI)	*P* value	IDI (%) (95% CI)	*P* value	Calibration, HLS *P* value
Stroke									
CHA_2DS_2–VASc	0.707 (0.621, 0.792)	<0.001	–	–	–	–	–	–	–
H19 + CHA_2DS_2–VASc	0.744 (0.661, 0.828)	<0.001	0.038 (0.006, 0.071)	0.022	71 (42.4, 76.4)	<0.001	1.2 (0.2, 9.6)	0.010	0.682
All-cause mortality									
CHA_2DS_2–VASc	0.658 (0.568, 0.748)	<0.001	–	–	–	–	–	–	–
H19 + CHA_2DS_2–VASc	0.684 (0.595, 0.773)	<0.001	0.026 (−0.008, 0.060)	0.138	21.8 (−12.1, 43.4)	0.129	2.5 (−0.2, 14.3)	0.109	0.228

CI: Confidence interval; HLS: Hosmer and Lemeshow goodness-of-fit test; IDI: Integrated discrimination improvement; NRI: Net reclassification improvement.



Risk score	AUC (95%CI)	*P* value (compared with CHA_2DS_2-VASc)
CHA_2DS_2-VASc	0.731 (0.661, 0.793)	–
H19+CHA_2DS_2-VASc	0.696 (0.624, 0.761)	0.059

Risk score	AUC (95%CI)	*P* value (compared with CHA_2DS_2-VASc)
CHA_2DS_2-VASc	0.687 (0.615, 0.752)	–
H19+CHA_2DS_2-VASc	0.666 (0.594, 0.733)	0.219

Figure 3: The ROC curves of the modified CHA_2DS_2–VASc score and the original score for the prediction of stroke and all-cause mortality. (A) For stroke, when adding H19 relative expression to CHA_2DS_2–VASc score, it showed a high discrimination performance with a borderline statically significant ($P = 0.059$). (B) For all-cause mortality, H19 could improve the discrimination performance of CHA_2DS_2–VASc score with no statistical difference. AUC: Area under curve; CI: Confidence interval; ROC: Receiver operating curve.

Discussion

To our knowledge, this is the first study to describe the association of high levels of plasma H19 with stroke and all-cause mortality risk in patients with AF. Our research revealed that the predictive ability of the CHA_2DS_2–VASc score for stroke was significantly improved when H19 expression was added.

The lncRNA H19 is encoded by the maternally imprinted gene, *H19*, which is located near the telomeric region of chromosome 11p15. It was identified and studied in mice by Pachnis *et al*[23] in 1984. Accumulative evidence has shown that lncRNA H19 plays a key role in the initiation, development, diagnosis, and prognosis of cardiovascular diseases. There has been evidence that H19 has a significant effect on the onset, development, and progression stages of atherosclerosis.[24] A study in an Iranian population found that circulating levels of H19 within 24 h of ischemic stroke onset could be used as an early diagnostic biomarker.[25] Wang *et al*[26] found that peripheral blood mononuclear cell-derived H19 was a risk factor for acute myocardial infarction and had significant diagnostic value.

Previous studies have revealed the link between lncRNA H19 and cardiac fibrosis; however, the results from different studies are controversial. Zhang *et al*[27] found significant downregulation of H19 expression after myocardial infarction and demonstrated by functional experiments that H19 reduced myocardial apoptosis and fibrosis and attenuated the inflammatory response. However, other researchers reported that H19 promotes cardiac fibrosis by targeting connective tissue growth factors as a miR-455 sponge.[28] An increasing number of

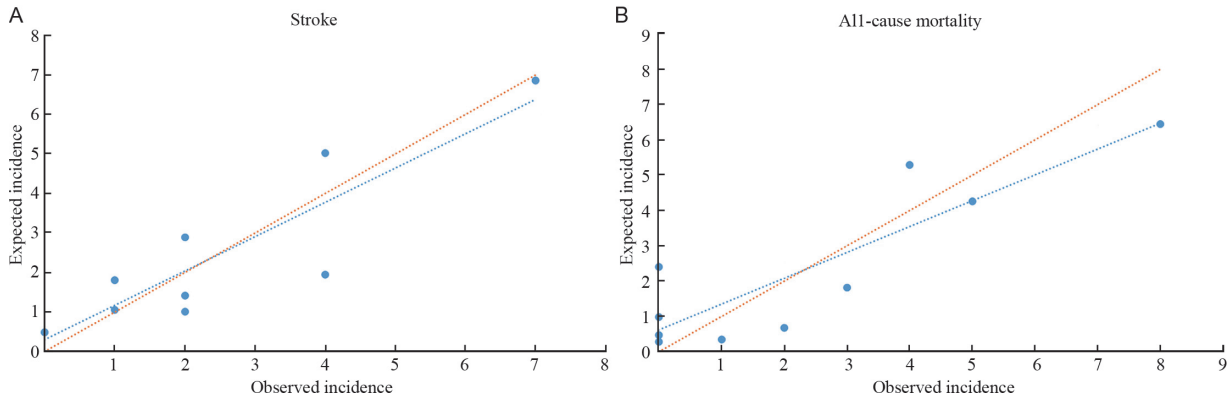


Figure 4: Calibration curve with H19 expression adding to CHA_2DS_2–VASc score for stroke and all-cause mortality. In this figure, we compared the predicted value (orange for CHA_2DS_2–VASc with H19 expression added) with the observed incidence (blue reference line). A shorter distance from the dots to the reference line indicates better calibration.

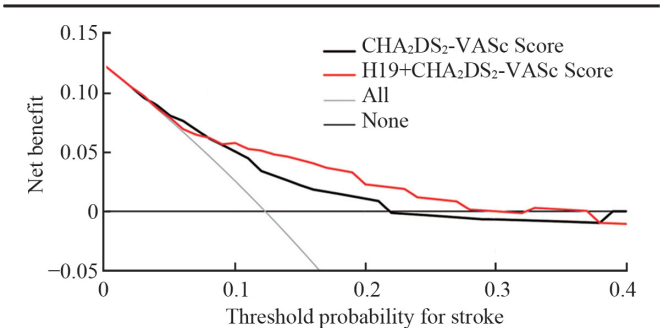


Figure 5: DCA for two scores. The X-axis indicates the threshold probability of the risk of the target adverse event and the Y-axis measures the net benefit. The black solid line represents the original CHA_2DS_2–VASc scores and the red solid line represents the modified CHA_2DS_2–VASc scores. DCA: Decision curve analysis.

evidence indicates that atrial fibrosis could be an important indicator of the severity and clinical prognosis of AF.[29] Therefore, we speculated that H19 may be involved in the development of AF by mediating myocardial fibrosis. However, the exact mechanism of our findings needs to be validated in further research.

Elevated plasma levels of H19 were shown to be an independent risk factor for stroke in our study. The method of using the expression level of long non-coding RNA in peripheral blood as a biomarker has the advantages of convenience and non-invasiveness, similar to findings in other studies.[30] The improvement in IDI and NRI of the CHA_2DS_2–VASc score with the addition of the new circulating biomarker H19 indicated that the new model had significantly improved predictive and net classification ability for stroke. The DCA provided insight into the predicted risk range of the models and showed higher clinical net benefits in favor of the new model.[22] Our data showed that the inclusion of H19 in the CHA_2DS_2–VASc score has a better net clinical benefit for patients with stroke.

However, we found no significant association of lncRNA H19 with all-cause mortality in either univariate or multivariable models. An elevated H19 level was associated with all-cause mortality with borderline significance (HR = 2.263, $P = 0.087$). The reason for this result may be due to the small sample size of our cohort and the limited number of all-cause mortality outcomes as the follow-up time was not long enough. Therefore, cohort studies with longer follow-up periods and larger sample sizes are needed to further validate our findings. In addition, it is imperative to point out that our research has another limitation. For people who took warfarin to prevent stroke, the guidelines recommended that time in therapeutic range (TTR) should be ≥70% to achieve the best clinical benefit.[31] Moreover, a lower TTR was significantly associated with stroke and all-cause mortality.[32] However, the data required to calculate the TTR were unfortunately unavailable in our retrospective cohort study. We cannot rule out the possibility that TTR might be a potential confounding factor in this study and could have had a non-negligible influence on the conclusions. Thus, the results of our study need external validation for further verify. Finally, selection bias might have been introduced because we recruited all AF patients from a single tertiary medical center.

Conclusion

In conclusion, elevated plasma H19 expression was an independent risk factor for stroke in patients with AF. Adding H19 levels could improve the prognostic value of the CHA_2DS_2–VASc score and increase net clinical benefits. LncRNA H19 may be a promising biomarker in the clinical decision-making of prognostic risk classification in patients with AF.

Conflicts of interest

None.

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