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RESEARCH ARTICLE

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Circulating long noncoding RNA, GAS5, as a novel biomarker for patients with atrial fibrillation

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Abstract

Background: Circulating long noncoding RNA (lncRNA) plays a vital role in clinical disease diagnosis and prognosis. Here, we evaluate the role of a lncRNA, named growth arrest specific 5 (GAS5), in atrial fibrillation (AF).

Methods: Expression of GAS5 was measured by qRT-PCR. Diagnostic and prognostic values of GAS5 were assessed by the receiver operating characteristics curve (ROC), Kaplan–Meier (KM) and Cox regression analyses.

Results: A total of 173 participants were enrolled in this study. Circulating GAS5 expression was significantly down-regulated in AF patients. This change occurred prior to enlargement of the left atrial volume and was strongly associated with AF progression, which demonstrates the potential use of GAS5 as an early biomarker. The area under the ROC curve (AUC) was 0.858 (95% CI 0.789-0.926, $P < .001$). Seventy of the 85 AF patients received radiofrequency catheter ablation (RFCA), and 22 (31.4%) had relapsed by the 1-year follow-up. The KM analysis (log-rank test, $P = .031$) and multivariable Cox analysis (HR = 0.127, 95% CI 0.026-0.616; $P = .01$) revealed that GAS5 has a role in predicting recurrence after RFCA.

Conclusion: Circulating lncRNA GAS5 is a potential biomarker for AF diagnosis and prognosis. Down-regulation of GAS5 occurs prior to left atrial enlargement and can be used for the prognosis of AF progression and recurrence.

KEYWORDS
atrial fibrillation, biomarker, GAS5, lncRNA, radiofrequency catheter ablation

1 | INTRODUCTION

Atrial fibrillation (AF) is one of the most common arrhythmias and presents a severe public health burden due to the accompanying risk of stroke and heart failure.[1,2] Strokes associated with AF are the third most common cause of all ischemic strokes and result in a higher level of disability and fatality compared with other causes, regardless of the AF type or burden.[3] In addition, long periods of out-of-control heart rate and rhythm can cause a deterioration in cardiac function, which leads to an increase in morbidity, mortality, and care burden, particularly in heart failure patients.[4] Currently, diagnosis of AF principally depends on electrocardiograms (ECG) or the use of a Holter monitor, within the AF duration,[5] which inevitably ignores patients whose AF attack is temporary and undetectable at certain points.

Radiofrequency catheter ablation (RFCA) is a common therapy for AF patients who are refractory to drug therapy as it helps to

maintain normal rhythm,[6] improves quality of life,[7] and reduces the risk of heart failure.[8] However, 25 to 40% of patients suffer from late recurrence after this procedure[6] and existing clinical indictors have limited value in predicting the outcome.

Long noncoding RNA (lncRNA) is defined as a transcript that is longer than 200 nucleotides, with no protein coding potential.[9] Generally, lncRNAs exhibit greater expression specificity and potential regulation ability, when compared with protein coding genes.[10] Recent studies have reported that a large number of lncRNAs have potential as biomarkers for diagnosis and prognosis in various cardiovascular diseases.[11] The level of a circulating lncRNA, UCA1, was reported to initially decline and then increase in the following 3 days in patients undergoing acute myocardial infarction, which indicates that it may be of diagnostic value.[12] The lncRNA, LIPCAR, was identified as a valuable biomarker for cardiac remodeling and also predicted the mortality of heart failure patients.[13] However, the evidence for the involvement of lncRNAs in AF is limited.

Growth arrest specific 5 (GAS5) is a lncRNA that accumulates in growth-arrested cells and alters cell susceptibility to apoptosis and other growth-related stimuli by modulating steroid hormone activity.[14] A microarray analysis recommended GAS5 as a novel biomarker due to its down-regulation in atrial tissues and the strong diagnostic power for AF displayed through the construction of an AF-related lncRNA-mRNA network (AFLMN).[15] Recently, Lu et al found a similar down-regulation of GAS5 in the right atrial appendage of AF patients.[16] Moreover, Tao et al reported that GAS5 suppressed cardiac fibroblast activation and fibrosis, which are crucial pathological features of AF, by the miR-21/PTEN axis.[17,18]

In this study, we aimed to explore the role of circulating GAS5 in both AF diagnosis and prognosis.

2 | METHODS

2.1 | Patients and controls

All AF patients were recruited between May and October 2018 and were diagnosed by ECG or a Holter monitor, in accordance with the latest guidelines. The exclusion criteria were as follows: (a) valvular heart disease or cardiomyopathy; (b) history of radiofrequency ablation; (c) history of acute myocardial infarction or angina; (d) left ventricular ejection fraction (LVEF) less than 50%; (e) cancer; (f) liver or renal failure; (g) uncontrolled hypertension or diabetes. The control group consisted of 43 people without AF or other organic heart diseases. In addition, 45 patients were selected as specific controls. These patients were diagnosed by ECG to have recently experienced paroxysmal supraventricular tachycardia (PSVT) but showed no evidence of AF. This study was approved by the Ethics Committee of the First Affiliated Hospital, Medical School of Zhejiang University, in accordance with the Declaration of Helsinki (2017721). All subjects signed the informed consent form to take part in this study.

2.2 | Definitions

In this study, AF patients were divided into four groups based on the left atrium volume index (LAVI), in accordance with the 2015 guideline for cardiac chamber quantification by echocardiograph. Normal size was a LAVI ≤ 34 mL/m^2; mildly abnormal was 34 < LAVI ≤ 41 mL/m^2; moderately abnormal was 41 < LAVI ≤ 48 mL/m^2; and severely abnormal was LAVI > 48 mL/m^2. Recurrence after RFCA was defined as any documented atrial arrhythmia (AF, atrial flutter or atrial tachycardia) lasting more than 30 seconds, after the 3-month blanking period. In accordance with the 2016 European Society of Cardiology (ESC) guidelines for the management of AF, patients with CHA2DS2-VASc scores of ≥1 in males or ≥2 in females were recommended for anticoagulation therapy.

2.3 | Plasma extraction

Whole blood samples (2 mL) were transferred into ethylenediaminetetraacetic acid (EDTA) anticoagulation tubes and centrifuged (1000 × g/min for 10 minutes at 4°C) to obtain the plasma. All samples were stored at −80°C in RNase-free microcentrifuge tubes.

2.4 | Total RNA isolation

Extraction of total RNA from plasma was performed using a miRNeasy Serum/Plasma Kit (QIAGEN, NO.217184), following the manufacturer's protocol. The GAPDH gene was selected as the internal control for sample variations. The RNA quantity and quality were measured using a Nanodrop 2000 (Thermo Scientific).

2.5 | Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

The total RNA was reverse-transcribed to cDNA with a PrimeScript RT reagent Kit (Takara), in accordance with the protocol, and subsequently quantified using TB Green Premix Ex Taq II (Takara) on a BIO-RAD CFX96™ Real-Time System. The reaction conditions were 95°C for 30 seconds, followed by 40 cycles of 95°C for 5 seconds and 60°C for 30 seconds. Relative expression of lncRNAs was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers used in this study are listed in Table S1.

2.6 | Radiofrequency catheter ablation (RFCA)

One day before the procedure, transesophageal echocardiography was performed to exclude the existence of left atrial appendage thrombus. All patients received radiofrequency-based circumferential pulmonary vein isolation (PVI). Additional procedures (ie, linear ablation and fragmentation potential ablation) were dependent on evaluation by the operator. Successful procedure was defined as an efferent block in all pulmonary veins and afferent block in all left atria, which were

TABLE 1 Characteristics of the study participants

	Control	PSVT	AF	*P*
N	43	45	85	–
Male	21 (48.84%)	22 (48.89%)	54 (63.53%)	.151
Age	61.07 ± 6.81	59.24 ± 8.27	60.35 ± 7.68	.526
BMI (kg/m^2)	24.14 ± 2.88	23.86 ± 3.08	25.71 ± 2.93^{a,bb}	.001
BSA (m^2)	1.68 ± 0.18	1.66 ± 0.14	1.78 ± 0.17^{aa,bb}	<.001
Smoking	8 (18.60%)	9 (20.00%)	28 (32.94%)	.123
Alcohol	10 (23.25%)	6 (13.33%)	28 (32.94%)	.047
Hypertension	21 (48.84%)	17 (37.78%)	43 (50.59%)	.362
Diabetes	3 (6.98%)	2 (4.44%)	11 (12.94%)	.237
Stroke	0 (0%)	1 (2.22%)	4 (4.71%)	.309
SP (mm Hg)	125 (21)	126 (20)	120 (18)	.063
DP (mm Hg)	78 (20)	77 (14)	76 (17)	.543
LAD (cm)	3.52 ± 0.39	3.24 ± 0.37^{a}	4.01 ± 0.50^{aa,bb}	<.001
LVEF (%)	68.25 ± 4.05	67.00 ± 4.94	65.15 ± 5.47^{aa}	.003
LVIDd (cm)	4.52 ± 0.38	4.55 ± 0.37	4.74 ± 0.38^{a,b}	.003
LAVI (mL/m^2)	–	27.45 ± 5.39	42.30 ± 11.64	<.001
LVMI (g/m^2)	–	94.24 (21.27)	99.68 (25.10)^{bb}	.001
hsCRP (mg/L)	1.17 (1.95)	0.67 (0.82)	0.81 (1.17)	.233
hsTnI (ng/mL)	0.004 (0.007)	0.003 (0.003)	0.003 (0.003)	.025
NT-proBNP (pg/mL)	18 (24.00)	24 (43.50)	85 (107.00)^{aa,bb}	<.001
Hyperuricemia (%)	10 (23.26%)	4 (8.89%)	23 (27.06%)	.052
TSH (mIU/L)	–	2.23 (1.78)	2.54 (2.16)	.582
Cystatin C (mg/L)	–	0.93 (0.17)	1.01 (0.27)^{bb}	.001
Cr (μmol/L)	71.77 ± 16.90	67.49 ± 11.57	74.89 ± 15.91^{b}	.025
TG (mmol/L)	1.55 (1.37)	1.37 (1.53)	1.62 (0.86)	.574
TC (mmol/L)	4.12 ± 1.11	4.38 ± 0.64	4.09 ± 0.97	.216
FBG (mmol/L)	5.01 (0.92)	4.57 (0.58)^{aa}	4.52 (1.06)^{aa}	.002

Note: Compared with control, $^{a}P < .05$; $^{aa}P < .01$; Compared with PSVT, $^{b}P < .05$; $^{bb}P < .01$.

Abbreviations: AF, atrial fibrillation; BMI, body mass index;BSA, body surface area; Cr, creatinine; DP, diastolic pressure; FBG, fasting blood glucose; hsCRP, hypersensitive C-reactive protein; hsTnI, hypersensitive troponin I; LAD, left atrium dimension; LAVI, left atrium volume index; LVEF, left ventricular ejection fraction; LVIDd, left ventricular diastolic dimension; LVMI, left ventricular mass index; NT-proBNP, N-terminal pro-B type natriuretic peptide; PSVT, paroxysmal supraventricular tachycardia; SP, systolic pressure; TC, total cholesterol; TG, triglyceride; TSH, thyroid-stimulating hormone.

reconfirmed 30 minutes after the last PVI. Details of the procedure can be obtained from a previous publication from our center.[19]

2.7 | Follow-up

Patients who received RFCA were followed up 3, 6, 9, and 12 months after the procedure. A 12-lead ECG and 24-hour Holter monitor were performed at each visit and at any other time when symptoms were present. Antiarrhythmic and anticoagulant drugs were prescribed for the first 3 months, and subsequent medication was prescribed based on assessment by the investigator, using recurrence and CHA2DS2-VASc scores.

2.8 | Statistical analysis

Descriptive statistics were presented as percentages for categorical variables and the mean ± SD or median (IQR) for continuous variables, dependent on normality. Categorical variables were analyzed using the χ^2 test. The Kolmogorov-Smirnov test was used to verify the normality of continuous variables. The Student's *t* test and one-way ANOVA were used for normally distributed data, while the Mann-Whitney *U* test and Kruskal-Wallis test were performed for abnormal variables. Spearman's rank correlation was used to explore the association between lncRNA and clinical characteristics of AF patients. Receiver operating characteristic curves (ROC) and area under the ROC curve (AUC) were constructed to

evaluate the efficiency of lncRNA in diagnosing AF. Age, sex, and variables with $P < .1$, in univariate logistic/Cox regression analyses, were selected for multivariate analysis to assess the diagnostic and prognostic value of lncRNA. Kaplan-Meier analysis was used to evaluate the cumulative recurrence rate during the 3 to 12 months after RFCA. We used SPSS 17.0 and GraphPad Prism 5.0 to perform the above analyses. A $P < .05$ was considered to be statistically significant.

3 | RESULTS

3.1 | Characteristics of study participants

We enrolled 85 AF patients and 43 controls in this study, in addition to 45 PSVT patients who were regarded as specific controls. Baseline characteristics of the three groups are summarized in Table 1. No statistically significant difference was observed in age or gender among the three groups, but the AF patients had higher body mass index, body surface area, hypersensitive troponin I levels, N-terminal pro-B type natriuretic peptide levels, cystatin C, and creatinine levels. The echocardiography information showed that AF patients exhibited a significant difference in left atrium dimension (LAD), left ventricular ejection fraction (LVEF), left atrium volume index (LAVI), left ventricular diastolic dimension (LVIDd), and left ventricular mass index (LVMI), when compared to the other groups.

3.2 | Screening of four selected lncRNAs in plasma of AF patients and controls

Four potential lncRNAs (NEAT1, GAS5, UCA1, and TUG1) were selected, in a screening trial, for their known role in cardiovascular diseases. The expression of these four candidates was measured by qRT-PCR performed on plasma from 20 AF patients and 20 controls. As shown in Figure 1, circulating GAS5 was decreased in AF patients, when compared with controls ($P < .001$), which shows its potential

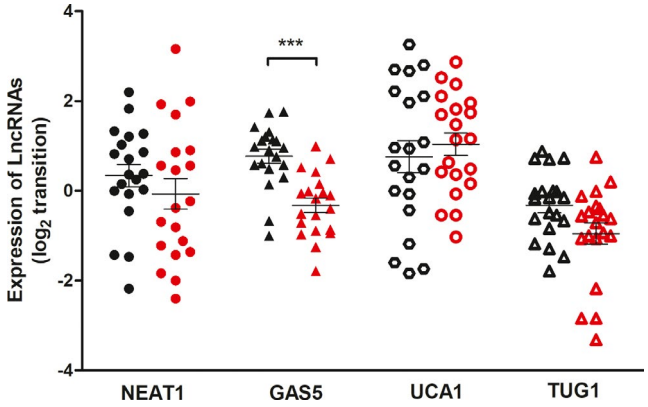

FIGURE 1 Validation of candidate lncRNAs (NEAT1, GAS5, UCA1 and TUG1) identified in screening study in plasma of AF patients and healthy controls. The expression levels of four candidate lncRNAs in 20 AF patients and 20 healthy controls were analyzed by qRT-PCR. *** means $P < .001$

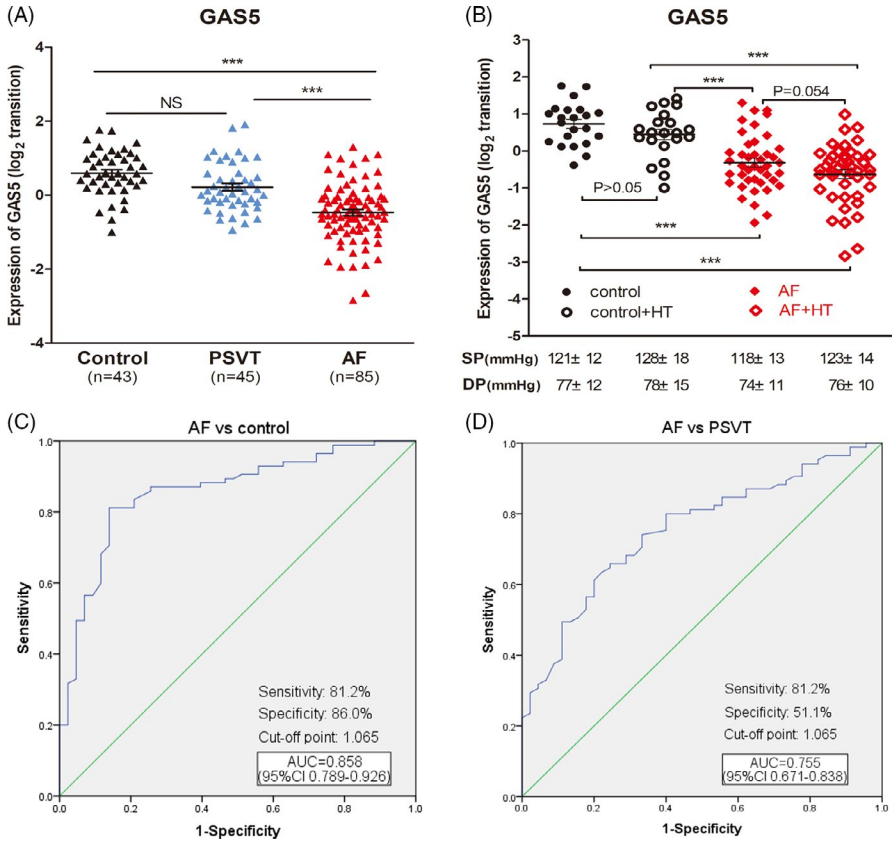

FIGURE 2 The diagnostic value of circulating GAS5 for AF patients. A, Eighty-five AF patients and 43 controls were enrolled in the study, with 45 PSVT patients selected as specific controls. B, Both AF patients and controls were divided into HT +/− subgroups. All AF subgroups had lower GAS5 expression when compared with control subgroups. No statistically significant difference was found between the two subgroups of the controls, whilst a weak significant difference was observed between HT ± AF patients. C, The value of GAS5 for AF diagnosis in AF patients when compared with the controls. D, The value of GAS5 for AF diagnosis in AF patients when compared with PSVT patients, using the same cut-off point. *** means $P < .001$

TABLE 2 Multivariate logistic analysis for the association of GAS5 with clinical characteristics

AF vs control				AF vs PSVT			
Characteristic	***P* value**	**OR**	**95%CI**	**Characteristic**	***P* value**	**OR**	**95%CI**
Age (y)	.882	1.009	0.902-1.128	Age (y)	.145	0.936	0.856-1.023
Sex (female)	.284	0.289	0.030-2.798	Sex (female)	.139	0.231	0.033-1.609
LAD (cm)	.437	1.998	0.349-11.428	LAVI (mL/m^2)	<.001	1.269	1.137-1.415
GAS5	.001	0.039	0.006-0.244	GAS5	.031	0.224	0.058-0.873
Smoking	.356	2.683	0.330-21.773	Alcohol	.008	0.062	0.008-0.479
BSA (m^2)	.131	679.06	0.145->999	Cr	.036	1.079	1.005-1.159
NT-proBNP (pg/mL)	.016	1.029	1.005-1.054	NT-proBNP (pg/mL)	.226	1.007	0.996-1.018
BMI (kg/m^2)	.415	0.866	0.612-1.225	BMI (kg/m^2)	.021	1.295	1.040-1.612
Thyroid dysfunction	.168	0.185	0.017-2.038	Thyroid dysfunction	.284	0.364	0.057-2.312

Note: Variates enrolled in multivariate analysis including age, sex, and variates with $P < .1$ in univariate analysis.
Abbreviation: OR, Odds ratio; thyroid dysfunction means subclinical or overt hyperthyroidism.

as a biomarker. No significant difference was observed in the other three lncRNAs ($P > .05$).

3.3 | Diagnostic value of circulating GAS5 for atrial fibrillation

In order to verify the expression of GAS5 in plasma, the 85 AF patients and 43 controls were used. As shown in Figure 2A, circulating GAS5 in AF patients was distinctly reduced when compared with the controls ($P < .001$). A ROC curve was constructed to evaluate the diagnostic value of GAS5 for AF (Figure 2C). The AUC was 0.858 (95% CI 0.789-0.926, $P < .001$), and at a cutoff point of 1.065, with 81.2% sensitivity and 86.0% specificity, the positive predictive value (PPV) was 92.0% and the negative predictive value (NPV) was 69.8%. Multivariate logistic regression analysis verified the independence of AF diagnosis. The odds ratio (OR) value of GAS5 was 0.039 (95% CI 0.006-0.244, $P = .001$) (Table 2).

3.4 | Influence of hypertension on GAS5 expression

To evaluate the influence of hypertension (HT) on GAS5 expression, both the AF and control groups were divided into HT +/−groups. The AF subgroups had lower GAS5 expression when compared with the control subgroups ($P < .001$). No statistically significant difference was found between the HT +/−control subgroups, while a weakly significant difference ($P = .054$) was observed between the AF subgroups (Figure 2B).

3.5 | Expression of GAS5 in patients with similar symptoms

Forty-five PSVT patients, who had similar symptoms (ie, palpitation and weakness) to AF, were enrolled to further verify the diagnostic value of GAS5. As shown in Figure 2A, the expression of GAS5 was decreased in AF patients, when compared with PSVT patients ($P < .001$). No difference was observed between controls and

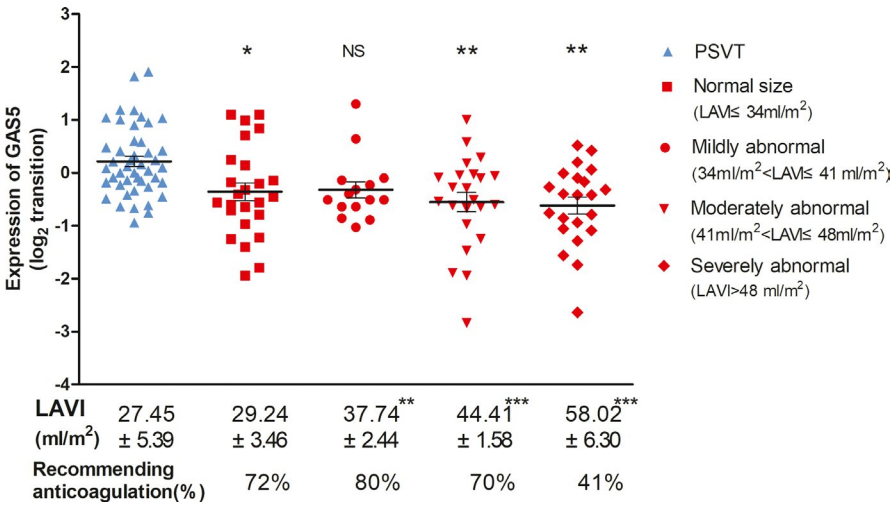

FIGURE 3 The expression of GAS5 in AF patients with different atrial sizes. AF patients were divided into four groups based on the left atrium volume index (LAVI). The HT rates among all groups showed no statistically significant differences. *$P < .05$; **$P < .01$; *** $P < .001$; all results were calculated using the PSVT group as the control

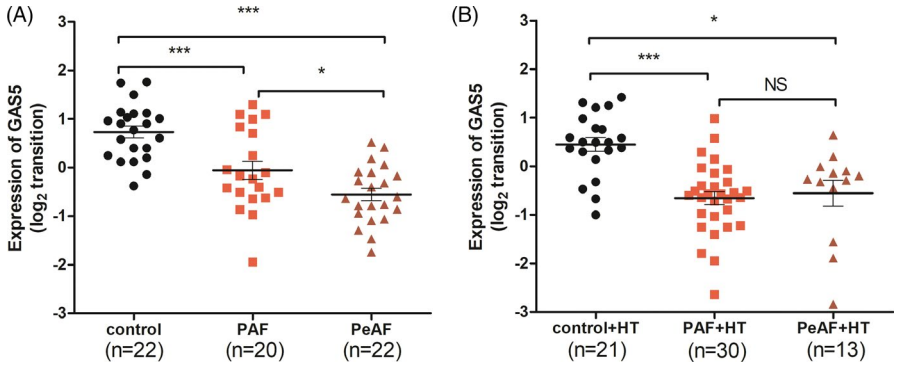

FIGURE 4 The expression of GAS5 in PAF and PeAF patients with or without HT. A, In HT- patients, PAF had a higher expression level of GAS5 when compared with PeAF. The levels of GAS5 expressed in PAF or PeAF patients were lower than in the controls. B, In HT+ patients, no statistically significant difference was observed between the PAF and PeAF groups. When compared with controls, the expression levels of GAS5 were reduced in PAF or PeAF patients. PAF, paroxysmal atrial fibrillation; PeAF, persistent atrial fibrillation; HT, hypertension; $^{*}P < .05$; $^{***}P < .001$

TABLE 3 Spearman's rank correlation analysis for the association of GAS5 with clinical characteristics

	Coefficient	*P*		Coefficient	*P*
AF risk factors					
Age	−.246	.023	Smoking	−.021	.846
Sex	−.051	.641	Alcohol	.129	.241
Hypertension	−.141	.200	BMI	−.041	.709
Diabetes	.036	.741	BSA	.048	.660
Cystatin C	−.250	.021	Cr	.060	.586
eGFR	.013	.906	TSH	.129	.241
Hyperuricemia	.009	.934			
Cardiac typical biomarkers					
hsTNI	−.182	.096	HBDH	.058	.599
AST	.078	.477	CK	.098	.371
LDH	.068	.537	CKMB	.185	.090
NT-proBNP	−.056	.609			
Echocardiography information					
LAD[a]	−.215	.051	LVEF	−.077	.486
LAV[a]	−.221	.044	LVIDd	.070	.526
LAVI[a]	−.215	.051			
Stroke risk score					
CHA2DS2-VASc	−.228	.036	CHA2DS2-VASc[b]	−.077	.487

Abbreviations: AF, atrial fibrillation; BMI, body mass index;BSA, body surface area; Cr, creatinine; DP, diastolic pressure; FBG, fasting blood glucose; hsCRP, hypersensitive C-reactive protein; hsTnI, hypersensitive troponin I; LAD, left atrium dimension; LAVI, left atrium volume index; LVEF, left ventricular ejection fraction; LVIDd, left ventricular diastolic dimension; LVMI, left ventricular mass index; NT-proBNP, N-terminal pro-B type natriuretic peptide; PSVT, paroxysmal supraventricular tachycardia; SP, systolic pressure; TC, total cholesterol; TG, triglyceride; TSH, thyroid-stimulating hormone; eGFR, Estimated glomerular filtration rate; AST, aspartate transaminase; LDH lactic dehydrogenase; HBDH, hydroxybutyrate dehydrogenase; CK, creatine kinase; CK-MB, creatine kinase-MB; LAV, left atrium volume.

[a]The coefficient and p value of LAD, LAV, and LAVI were analyzed "after controlling the factors including age and hypertension."

[b]The coefficient and p value of CHA2DS2-VASc score were analyzed "after controlling the age."

PSVT patients ($P > .05$). Taking 1.065 as the cutoff point, as calculated above, the sensitivity was 81.2% and specificity was 51.1%, with a PPV of 75.82% and NPV of 58.97% (Figure 2D). Multivariate analysis verified the independence of GAS5, and the OR value was 0.224 (95% CI 0.058-0.873, $P = .031$; Table 2). To explore the influence of left atrial volume on GAS5 expression, all AF patients were

TABLE 4 Characteristicsof AF patients who received RFCA

	No recurrence (n = 48)	Recurrence (n = 22)	*P*
Age	59.13 ± 8.32	59.91 ± 6.06	.694
Male	31 (64.58%)	16 (72.73%)	.501
Smoking	16 (33.33%)	8 (36.36%)	.804
Drinking	16 (33.33%)	9 (40.91%)	.539
Persistent AF	17 (35.42%)	12 (54.55%)	.131
Hypertension	22 (45.83%)	10 (45.45%)	.976
Diabetes	8 (16.67%)	1 (4.55%)	.160
Stroke	2 (4.17%)	0 (0.00%)	.331
BMI (kg/m^2)	25.61 ± 3.04	25.65 ± 2.90	.956
BSA (m^2)	1.77 ± 0.15	1.80 ± 0.21	.485
CHA2DS2-VASc score	1 (1)	1 (2)	.500
LAD (cm)	3.93 ± 0.55	4.18 ± 0.39	.056
LAVI (mL/m^2)	40.17 ± 10.40	46.48 ± 14.20	.040
LVEF (%)	65.60 ± 4.71	64.51 ± 6.09	.418
LVIDd (cm)	4.67 ± 0.31	4.87 ± 0.39	.022
LVMI (g/m^2)	101.32 ± 19.40	110.68 ± 23.11	.103
hsCRP (mg/L)	1.46 (1.02)	3.07 (1.32)	.058
NT-proBNP	103.54 (123.00)	128.50 (107.75)	.061
Cr(μmol/L)	74.67 ± 13.51	75.27 ± 18.43	.877

divided into four groups based on the LAVI. No difference in HT rate ($P > .05$) was observed among the groups. We found that GAS5 was down-regulated in AF patients with a normal size left atrium, when compared to PSVT patients (Figure 3).

3.6 | Expression of GAS5 in atrial fibrillation with different progression

The AF patients were divided into two groups, paroxysmal and persistent, based on disease progression. The imbalanced distribution of HT in these two groups was considered (60.00% vs. 37.14%, respectively, $P = .038$), and all participants were initially divided into HT +/−groups. We found that both paroxysmal AF (PAF) and persistent AF (PeAF) patients had lower GAS5 levels, when compared to the controls, regardless of HT status. In the HT- patients, the PeAF group had lower expression of GAS5 when compared with the PAF group ($P < .05$), while no statistically significant difference was observed in the HT+ patients (Figure 4). These results showed that GAS5 was further down-regulated in PeAF patients without HT.

3.7 | Association between GAS5 and clinical characteristics in atrial fibrillation

Table 3 shows the association between GAS5 and clinical characteristics of AF patients. The data demonstrated that GAS5 was negatively correlated with age, cystatin C, and CHA2DS2-VASc score ($P < .05$). After adjustment for the interference of age, no correlation was observed between GAS5 and the CHA2DS2-VASc score ($P = .487$). To evaluate the influence of age and HT on atrial size, we adjusted these two variables and found a roughly negative correlation between GAS5 and certain echocardiography indices, which included LAD ($P = .051$), LAV ($P = .044$), and LAVI ($P = .051$).

3.8 | Prognostic value of circulating GAS5 for post-ablation recurrence

Seventy patients who were resistant to antiarrhythmic drugs received RFCA. At the 1-year follow-up, 22 patients (31.4%) had relapsed with a larger LAVI ($P = .04$) and LVIDd ($P = .022$) (Table 4). The Kaplan-Meier analysis revealed that the lower GAS5 group had a higher risk of relapse after RFCA (log-rank test, $P = .031$) (Figure 5A). Multivariate analysis showed that GAS5 (Hazard Ratio (HR)=0.127, 95% CI 0.026-0.616, $P = .01$) and LVIDd (HR = 1.191, per 0.1 cm increase, 95% CI 1.052-1.348, $P = .006$) were independent predictors of recurrence, after adjustment for age, sex, LAD, LAVI, LVMI, and hypersensitive C-reactive protein (hsCRP) (Table 5).

4 | DISCUSSION

To date, lncRNAs have been used as novel biomarkers in many fields, such as cardiovascular diseases (CVD).[12,20] Growth arrest specific 5 is found at high levels in growth-arrested cells and functions as a riborepressor of the glucocorticoid receptor (GR). It binds to the glucocorticoid response element sequence to suppress the transcription of the GR and thereby modulates cell growth and metabolism.[14] Previous studies have confirmed the stability of GAS5 expression in plasma.[21] In CVD, studies have reported that GAS5 may be a promising biomarker for HT and coronary artery disease.[22,23] A recent microarray analysis recommended GAS5 as a novel biomarker in atrial tissues, in which it displays strong diagnostic power for AF. This was observed through the construction of an AF-related lncRNA-mRNA network (AFLMN). Functional module analysis has revealed that GAS5 is closely associated with 52 mRNAs and is enriched in many AF-associated pathways involved in inflammation, ion channel, and metabolism regulation.[15] Another group has also confirmed a significant difference in GAS5 expression in atrial tissue from AF patients and has highlighted its inhibiting effect on proliferation.[16]

Hypertension is the most common complication in AF patients. In this study, 50.59% of AF patients experienced HT, which is consistent with previously reported data.[24] Recent research has suggested that the down-regulation of GAS5 in HT patients indicates the role of GAS5 in HT progress.[22] Researchers found that electroanatomic variations, which were attributed to HT in PSVT patients, promote the susceptibility and duration of AF. These electroanatomic variations were also discovered in AF patients with HT, which suggests that HT facilitates the progress of AF.[25] In our study, no difference was observed in GAS5 expression between controls divided into

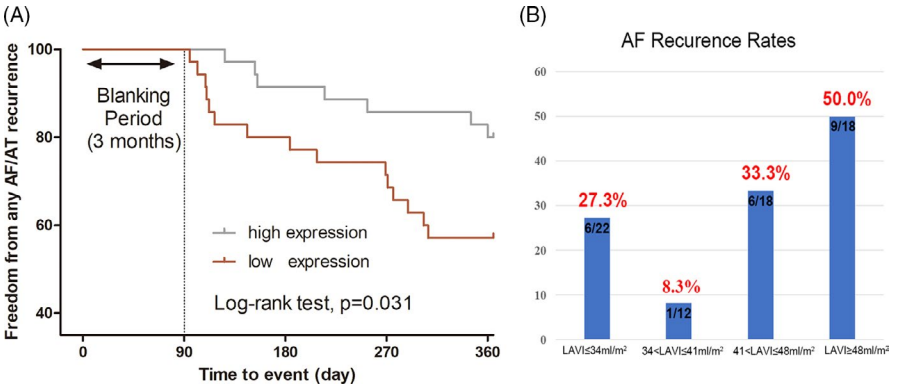

FIGURE 5 A, The Kaplan-Meier analysis of freedom from AF/AT recurrence during the 12 months after RFCA, with high or low levels of circulating GAS5. B, Different AF recurrence rates among patients with different size atria. AT, atrial tachycardia

TABLE 5 Univariable and multivariable Cox regression of AF recurrence

	Univariable			Multivariable		
Characteristics	***P* value**	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**
Age	0.754	1.009	0.956-1.064	.811	1.008	0.941-1.081
Male	0.530	1.351	0.528-3.453	.805	1.135	0.415-3.109
GAS5	0.017	0.148	0.031-0.706	.010	0.127	0.026-0.616
Smoking	0.747	1.154	0.484-2.750			
Drinking	0.577	1.274	0.544-2.980			
Persistent AF	0.110	1.987	0.857-4.608			
Hypertension	0.943	0.970	0.419-2.245			
Diabetes	0.240	0.300	0.040-2.235			
Stroke	0.560	0.047	0.000->999			
BMI	0.928	1.006	0.875-1.158			
BSA, per 0.1 m^2 increase	0.527	1.085	0.843-1.396			
LAD	0.067	2.180	0.948-5.013	.958	1.034	0.297-3.602
LAVI	0.024	1.041	1.005-1.078	.119	1.030	0.992-1.069
LVEF	0.293	0.956	0.879-1.040			
LVIDd, per 0.1 cm increase	0.011	1.175	1.037-1.332	.006	1.191	1.052-1.348
LVMI	0.041	1.020	1.001-1.040	.706	1.005	0.980-1.031
hsCRP	0.085	1.055	0.993-1.122	.338	1.040	0.960-1.126
NT-proBNP	0.303	1.002	0.998-1.005			
Hyperuricemia	0.567	1.299	0.530-3.188			
Cr	0.924	0.999	0.971-1.027			
Thyroid dysfunction	0.976	0.985	0.363-2.670			

Abbreviations: AF, atrial fibrillation; BMI, body mass index;BSA, body surface area; Cr, creatinine; DP, diastolic pressure; FBG, fasting blood glucose; HR, hazard ratio; hsCRP, hypersensitive C-reactive protein; hsTnI, hypersensitive troponin I; LAD, left atrium dimension; LAVI, left atrium volume index; LVEF, left ventricular ejection fraction; LVIDd, left ventricular diastolic dimension; LVMI, left ventricular mass index; NT-proBNP, N-terminal pro-B type natriuretic peptide; PSVT, paroxysmal supraventricular tachycardia; SP, systolic pressure; TC, total cholesterol; TG, triglyceride; TSH, thyroid-stimulating hormone.

the HT +/−subgroups, but a weak down-regulation of GAS5 was found in AF patients with HT (P = .054). This was not consistent with the original data, which may be partly attributed to a better management of blood pressure in this study.[22] The interaction of these two diseases may promote the decrease of GAS5. It is worth noting that a lower cutoff point should be used for AF patients with uncontrolled HT.

All AF patients were divided into PAF and PeAF subgroups. The PAF group always experienced a lower AF burden and had a higher rate of success for conversion when compared with the

PeAF group. After 10 years of follow-up, more than 50% of the PAF group had progressed to persistent, but no significant difference was observed between stroke and AF type.[26] Furthermore, the PeAF group was characterized by more serious electrical and structural remodeling, which are primary pathophysiological variations for AF recurrence.[27] Tao et al proved that GAS5 suppresses cardiac fibroblast activation and fibrosis by the miR-21/PTEN/MMP-2 axis, which are hallmarks of AF structural remodeling, characterized by the down-regulation of Type I collagen (Col1A1) and smooth muscle alpha-action (α-SMA) expression.[17,18] Recently, this group has also reported DNA methylation alterations of GAS5 in cardiac fibrosis.[28] Liu et al observed a marked down-regulation of GAS5 in a cardiac fibrosis animal model, which was induced by isoproterenol, and demonstrated that overexpression of GAS5 attenuates this pathological process.[29] Altogether, this evidence indicates that GAS5 plays a crucial role in AF structural remodeling. Our studies confirmed a further down-regulation of circulating GAS5 in PeAF patients with no history of HT ($P < .05$), which demonstrated that the level of GAS5 may be used for the prognosis of AF progression.

Seventy of 85 AF patients received RFCA, and 22 had relapsed at the 1-year follow-up. We confirmed the value of circulating GAS5 for the prediction of AF recurrence. Left atrium volume index (LAVI) reflects the degree of structural remodeling and has been regarded as an ideal marker for prognosis and stratification of cardiovascular risks, such as AF. One study followed up a random sample of 1655 patients and proved that larger left atrial volume accompanied a higher risk of AF.[30] In our study, AF patients were divided into four groups, according to the LAVI. Results showed that circulating GAS5 decreased prior to atrial enlargement, which reveals its advantage over LAVI in recognizing patients who are characterized by normal atria but are at a similar risk of stroke. Furthermore, LAVI was also considered to be a potential indicator for prediction of the recurrence of AF, but the data have been inconsistent.[31,32] In our study, LAVI was not an independent factor for the prognosis of recurrence. Our center has previously reported a U-shaped relationship between LAD and AF recurrence, rather than a linear trend. This relationship indicated that medium-sized atria were related to a lower recurrence rate,[19] which is consistent with the results from our study.

There were some limitations to our study. Firstly, a limited selection of lncRNA markers was assessed. We expect that many other lncRNAs are regulated in AF and may act as biomarkers. Secondly, the number of samples in our study was small and monocentric. Large-scale and multicentric trials are needed to further verify the role of GAS5 as a biomarker, for both diagnosis and prognosis.

5 | CONCLUSIONS

In conclusion, the circulating lncRNA, GAS5, is a potential biomarker for AF diagnosis and prognosis. The down-regulation of GAS5, prior to left atrial enlargement, shows that it can be used for the prognosis of AF progression and recurrence.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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