

Circulating Long Noncoding RNA GAS5 as a novel biomarker for patients with atrial fibrillation

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Abstract

Background Long noncoding RNA (LncRNA) played a vital role in pathophysiology of cardiovascular diseases. However, its role in the diagnosis of atrial fibrillation (AF) remains unknown. The aim of this study is to identify the diagnostic value of LncRNA GAS5 for AF patients.

Methods Four LncRNAs (NEAT1, GAS5, UCA1, and TUG1) were selected as potential biomarkers of AF. The circulating expression of LncRNAs were measured by qRT-PCR. Receiver operating characteristic curve (ROC) and area under the ROC curve (AUC) were applied to assess their diagnostic value for AF.

Results In screening trial, LncRNA GAS5 was down-regulated in AF patients, with no significant differences in other three LncRNAs. Then a total of 128 participants were enrolled including 85 AF patients and 43 controls. Circulating levels of GAS5 in AF patients were remarkably reduced compared with controls ($P < 0.001$). The AUC was 0.858, with 81.2% sensitivity and 86.0% specificity. Further, the downregulation of GAS5 was more significant in persistent rather than paroxysmal AF. Correlation analysis showed that GAS5 was negatively correlated with CHA2DS2-VASc score and several echocardiography indexes.

Conclusions Circulating LncRNA GAS5 is a potential biomarker for AF diagnosis and may prognose AF progression and stroke risk.

Background

Atrial fibrillation (AF) is one of the most common arrhythmia, accompanying with severe public health burden for its risk of stroke and heart failure. From 1990 to 2010, the mortality associated with AF had 2-fold and 1.9-fold increases in men and women respectively[1]. In the United States, the estimated incremental cost of AF patients is \$26.0 billion in 2010[2] and the prevalence will rise to 15.9 million by 2050, which means a 3-fold care burden[3]. In recent years, more and more elders suffered from atrial fibrillation. And the stroke associated with AF was characterized as higher disabling and fatality[4]. In addition, long periods of out-of-control rate and rhythm could deteriorate cardiac function causing increased morbidity, mortality and care burden, especially in heart failure patients[5]. Currently, the diagnosis of AF principally depends on electrocardiogram (ECG) or Holter monitor within AF duration[6]. But these finite monitoring inevitably ignored a mass of AF patients whose abnormal ECG were temporary and undetectable at certain points. Therefore, newly stable biomarkers are needed to improve the [efficiency](#) of AF diagnosis.

Long non-coding RNA (LncRNA) is defined as transcripts which is longer than 200 nucleotides with no protein coding potential[7]. They exhibited greater expression specificity and potential regulation ability compared with protein coding genes[8]. Recent studies had reported that a great number of LncRNAs functioned as [indispensable](#) molecules and ideal biomarkers of diagnosis and prognosis in various cardiovascular diseases[9, 10]. However, the evidence of LncRNAs in atrial fibrillation is limited.

Therefore, several potential lncRNAs were selected considering their role in cardiovascular diseases.

Growth arrest specific 5 (GAS5) accumulates in growth-arrested cells and alters cell susceptibility to apoptosis and other growth-related stimuli by modulating steroid hormone activity[11]. In cardiovascular disease, hypertension (HT) was the first member to be reported. Researchers observed the distinct downregulation of GAS5 in HT patient and models' plasma as compared to controls and identified GAS5 as a potential regulator in HT-related vascular remodeling[12]. Interestingly, similar downregulation of GAS5 in plasma was proved in coronary artery disease showing satisfactory sensitivity and specificity[13]. Moreover, Tao et al. reported that GAS5 suppressed cardiac fibroblast activation and fibrosis by miR-21/PTEN axis[14], which was crucial pathological features of AF[15].

Nuclear paraspeckle assembly transcript 1 (NEAT1) had been regarded as a critical biomarker in various carcinomas[16, 17]. In addition, NEAT1 participated in the process of vascular smooth muscle cell phenotypic switching[18] and acted as a key modulator in immune cells which was inhibited in myocardial infarction[19].

Taurine Upregulated Gene 1 (TUG1) regulated cardiac fibroblast-myofibroblast transformation in chronic hypoxia demonstrating its probable function in cardiac fibrosis[20]. As a vital regulator, TUG1 was also involved in vascular smooth muscle cell dysfunction in HT and atherosclerosis model[21, 22].

Urothelial carcinoma associated 1 (UCA1) gene belongs to the HERV-H family, had been regarded as an ideal biomarker to diagnose bladder cancer[23]. In cardiomyocytes, UCA1 suppressed cell apoptosis through regulating the expression of p27 in ischemia reperfusion injury model[24]. What's more, Circulating UCA1 had been reported to decline first, and increase in later 3 days in patients undergoing acute myocardial infarction, indicating its promising diagnosis value[25].

In this study, we aimed to explore the expression of four lncRNAs in atrial fibrillation patients and confirmed their capacity as applicable biomarkers.

Methods

Patients and controls

All atrial fibrillation patients were recruited between May 2018 and October 2018 and diagnosed by ECG or Holter monitor according to latest guideline. The exclusion criterias of subject list as follows: (1) Valvular heart disease or cardiomyopathy; (2) history of radiofrequency ablation; (3) history of acute myocardial infarction or angina; (4) left ventricular ejection fraction (LVEF) less than 50%; (5) cancer; (6) liver or renal failure; (7) uncontrolled hypertension or diabetes. And the control group incorporated 43 persons without AF or other organic heart diseases. Additionally, 45 patients who experienced paroxysmal supraventricular tachycardia (PSVT) recently and diagnosed by ECG with no experience of AF were selected as specific controls.

Definitions

In accordance with 2016 ESC guidelines for the management of atrial fibrillation, AF was divided into paroxysmal and persistent. Paroxysmal AF (PAF) was defined as an episode of AF recorded by ECG or Holter with the ability of reversing to sinus rhythm spontaneously in 7 days. Persistent AF (PeAF) was defined as an episode of AF diagnosed by ECG or Holter which lasted longer than 7 days. The diagnosis of PSVT relied on ECG recorded at PSVT onset and the following electrophysiological examination. What's more, patients whose CHA₂DS₂-VASc score was ≥ 1 in male or ≥ 2 in female were recommended to receive anticoagulation therapy according to guideline above. CHA₂DS₂-VASc score includes Congestive Heart failure, hypertension, Age ≥ 75 (doubled), Diabetes, Stroke (doubled), Vascular disease, Age 65–74, and Sex (female).

Plasma extraction

Peripheral venous blood (2ml) was transferred into Ethylenediaminetetraacetic acid (EDTA) anticoagulated tubes and centrifuged (1000g for 10 minutes at 4 °C) to gain plasma. All samples were stored at –80 °C until use.

Total RNA isolation

Extraction of total RNA from plasma was performed using miRNeasy Serum/Plasma Kit (QIAGEN) in line with the protocol. The concentration of RNA was measured by Thermo Scientific Nanodrop 2000.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

The total RNA was reverse-transcribed to cDNA with PrimeScript RT reagent Kit (Takara) in accordance with protocol and subsequently quantified by TB Green Premix Ex Taq II (Takara) performing on BIO-RAD CFX96™ Real-Time System. The reactions were at 95°C for 30s, followed by 40 cycles of 95°C for 5s and 60°C for 30s. Relative expression of LncRNAs were calculated by $2^{-\Delta\Delta Ct}$.

The primers used in this study are listed in supplemental document.

Statistical analysis

Descriptive statistics were presented as percentage for categorical variables and mean \pm SD for continuous variables. Categorical variables were analyzed using χ^2 test. Kolmogorov –Smirnov test was used to verify the normality of continuous variables. Student's *t*-test and one-way ANOVA were suitable for normal distributed data, while Mann-Whitney U test and Kruskal-Wallis test were performed for

abnormal variables. Spearman rank correlation was used to explore the association between LncRNA and clinical characteristics of AF patient. Age, sex and variables with $P < 0.1$ on univariate logistic regression analysis were selected for multivariate analysis to assess the predictive value of LncRNA. Receiver operating characteristic curves (ROC) and area under the ROC curve (AUC) was constructed to evaluate the efficiency of LncRNA in diagnosing AF. Using SPSS 17.0 and GraphPad Prism 5.0 to accomplish analysis. $P < 0.05$ was considered statistical significance.

Results

Characteristics of study objects

85 AF patients and 43 controls were enrolled in this study, as well as 45 PSVT patients regarded as specific controls. Baseline characteristics of three groups were summarized in Table 1. No statistic difference was observed in age or gender among three groups, but AF patients had higher BMI, BSA, hsTnl, NT-proBNP, Cystatin C and Cr. As for echocardiography information, AF patients exhibited significantly difference in LAD, LVEF, LAVI, LVMI and so on, indicating their structural remodeling. Further, we investigated the features of PAF and PeAF patients which listed in Table 2. Data showed higher HT rate and CHA2DS2-VASc scores in PAF patients with matched age and gender compared with PeAF. Meanwhile, PAF had smaller LAD and LAVI, higher LVEF and lower level of TG.

Table 1 Characteristics of the study participants

	Control	PSVT	AF	P
Clinical characteristic				
N	43	45	85	-
Male (%)	21(48.84%)	22(48.89%)	54(63.53%)	0.151
Age (years)	61.07±6.81	59.24±8.27	60.35±7.68	0.526
BMI (kg/m ²)	24.14±2.88	23.86±3.08	25.71±2.93 ^{a,bb}	0.001
BSA (m ²)	1.68±0.18	1.66±0.14	1.78±0.17 ^{aa,bb}	<0.001
Smoking (%)	8(18.60%)	9(20.00%)	28(32.94%)	0.123
Alcohol (%)	10(23.25%)	6(13.33%)	28(32.94%)	0.047
Hypertension (%)	21(48.84%)	17(37.78%)	43(50.59%)	0.362
Diabetes (%)	3(6.98%)	2(4.44%)	11(12.94%)	0.237
Stroke (%)	0(0%)	1(2.22%)	4(4.71%)	0.309
SP (mmHg)	124.72±15.50	126.18±13.66	120.55±13.47	0.063
DP (mmHg)	77.16±13.62	77.00±9.26	75.22±10.46	0.543
Echocardiography information				
LAD (cm)	3.52±0.39	3.24±0.37 ^a	4.01±0.50 ^{aa,bb}	<0.001
LVEF (%)	68.25±4.05	67.00±4.94	65.15±5.47 ^{aa}	0.003
LVIDd (cm)	4.52±0.38	4.55±0.37	4.74±0.38 ^{a,b}	0.003
LAVI (ml/m ²)	-	27.45±5.39	42.30±11.64	<0.001
LVPWd (cm)	-	0.95±0.11	1.01±0.09 ^b	0.012
IVSd (cm)	-	1.03±0.10	1.09±0.10 ^{bb}	0.003
LVPWs (cm)	-	1.39±0.17	1.46±0.18 ^b	0.037
LVM (g)	-	154.41±29.54	186.66±39.34 ^{bb}	<0.001
LVMi (g/m ²)	-	92.84±15.87	105.00±20.03 ^{bb}	0.001
LVIDs (cm)	-	2.85±0.28	3.03±0.31 ^{bb}	0.002
IVSs (cm)	-	1.43±0.13	1.53±0.17 ^{bb}	0.001
Traditional biomarker				
hsCRP (mg/L)	1.77±2.09	1.64±3.49	1.90±4.07	0.233
hsTnI (ng/ml)	0.009±0.012	0.004±0.004	0.011±0.055 ^b	0.025
NT-proBNP (pg/ml)	27.65±29.20	42.24±40.15	124.87±172.05 ^{aa,bb}	<0.001
Hyperuricemia (%)	10(23.26%)	4(8.89%)	23(27.06%)	0.052
Cystatin C (mg/L)	-	0.96±0.14	1.08±0.23 ^{bb}	0.001
eGFR (ml/min)	86.62±14.75	93.08±10.78	87.43±13.65 ^b	0.019
Cr (μmol/L)	71.77±16.90	67.49±11.57	74.89±15.91 ^b	0.025
TG (mmol/L)	2.06±1.71	1.75±1.11	1.80±0.99	0.574
TC (mmol/L)	4.12±1.11	4.38±0.64	4.09±0.97	0.216
TSH (mIU/L)	-	2.53±1.53	2.94±2.29	0.582
FBG (mmol/L)	5.30±1.52	4.64±0.81 ^{aa}	4.79±1.28 ^{aa}	0.002

Compared with control, ^a P<0.05; ^{aa} P<0.01; Compared with PSVT, ^b P<0.05; ^{bb} P<0.01.

Table 2 Characteristics of AF patients in different type.

	Paroxysmal AF	Persistence AF	P
Clinical characteristic			
N	50	35	-
Male (%)	30(60.00%)	24(68.57%)	0.419
Age	61.68±7.44	58.46±7.74	0.057
BMI (kg/m ²)	25.29±3.01	26.32±2.74	0.113
BSA (m ²)	1.77±0.16	1.79±0.19	0.691
Smoking (%)	12(24.00%)	16(45.71%)	0.036
Alcohol (%)	14(28.00%)	14(40.00%)	0.247
Hypertension (%)	30(60.00%)	13(37.14%)	0.038
Diabetes (%)	9(18.00%)	2(5.71%)	0.097
Stroke (%)	3(6.00%)	1(2.86%)	0.640
CHADS ₂ score	0.94±0.94	0.60±0.775	0.064
CHA ₂ DS ₂ -VASc score	1.78±1.31	1.11±1.21	0.006
Recommending anticoagulation [#] (%)	38(76.00%)	17(48.57%)	0.009
Echocardiography information			
LAD (cm)	3.83±0.44	4.26±0.49	∞0.001
LVEF (%)	66.67±4.80	62.97±5.68	0.002
LVIDd (cm)	4.71±0.38	4.77±0.39	0.491
LAVI (ml/m ²)	36.91±7.85	49.99±11.93	∞0.001
LVPWd (cm)	1.00±0.094	1.01±0.093	0.911
IVSd (cm)	1.10±0.11	1.07±0.089	0.182
LVPWs (cm)	1.46±0.17	1.45±0.20	0.819
LVM (g)	180.19±33.97	195.91±44.84	0.069
LVMI (g/m ²)	101.81±17.47	109.56±22.68	0.079
LVIDs (cm)	2.97±0.27	3.12±0.35	0.024
IVSs (cm)	1.53±0.18	1.54±0.15	0.803
Traditional biomarker			
Cr (μmol/L)	74.60±14.98	75.31±17.37	0.840
eGFR (ml/min)	87.09±11.44	87.92±16.47	0.414
TG (mmol/L)	1.64±0.95	2.02±1.01	0.032
TC (mmol/L)	4.02±1.04	4.19±0.88	0.416

[#]According to 2016 ESC guideline of AF, CHA₂DS₂-VASc score ≥1 in male or ≥2 in female was recommended to receive anticoagulation therapy. CHA₂DS₂-VASc including Congestive Heart failure, hypertension, Age ≥75 (doubled), Diabetes, Stroke (doubled), Vascular disease, Age 65-74, and Sex (female).

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

The expression of four potential lncRNAs (NEAT1, GAS5, UCA1, and TUG1) between AF patients and controls were measured by qRT-PCR first. As shown in Figure 1, Circulating GAS5 was evidently decreased in AF patients compared with controls ($P < 0.001$) showing its potential as a biomarker. No significant differences in other three lncRNAs were observed ($P > 0.05$).

The influence of hypertension in GAS5 expression

In order to verify the influence of HT in GAS5 expression, AF, PSVT patients and controls were divided into two within groups first according to whether they have HT or not. And a dubious decrease of GAS5 expression was found in HT subgroup without statistical significance (Figure 2A). What's more, AF patient accompanied with HT was separately compared with AF or HT patients. Results shown a convictive significance between HT patients with AF or not ($P < 0.01$) (Figure 2B). Our date confirmed a weak down-regulation of GAS5 in HT subgroups, and further decrease was observed in HT patients with AF.

GAS5 as a biomarker between AF patients and controls

85 AF patients and 43 control were enrolled in this study. In Figure 2A, the circulating expression of GAS5 in AF patients distinctly decreased compared with controls ($P < 0.001$). ROC curve was constructed to evaluate diagnosis value of GAS5 for AF (Figure 3A). The area under ROC curve (AUC) was 0.858 (95%CI 0.789–0.926, $P < 0.001$) as compared to controls, and at a cut-off point at 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 its independence in AF diagnosis. The OR values of GAS5 were 0.039 (95%CI 0.006–0.244, $P = 0.001$) (Table 3). These results displayed attractive diagnostic efficiency and independent predicting value of GAS5 for AF.

Table 3 Multivariate logistic analysis for the association of GAS5 with clinical characteristics between AF patients and controls.

	P value	Odds ratio	95% Confidence interval
Age (years)	0.882	1.009	0.902-1.128
Sex (female)	0.284	0.289	0.030-2.798
LAD (cm)	0.437	1.998	0.349-11.428
GAS5	0.001	0.039	0.006-0.244
Smoking	0.356	2.683	0.330-21.773
BSA(m ²)	0.131	679.058	0.145->999
NT-proBNP (pg/ml)	0.016	1.029	1.005-1.054
BMI (kg/m ²)	0.415	0.866	0.612-1.225
Thyroid dysfunction	0.168	0.185	0.017-2.038

Thyroid dysfunction means Subclinical or overt hyperthyroidism.

Expression of GAS5 in diseases with similar symptoms

In addition, 45 PSVT patients who had similar symptoms to AF were enrolled to further verify the diagnostic value of GAS5. As shown in Figure 2A, the expression of GAS5 was similarly decreased in AF patients compared with PSVT patients ($P < 0.001$). And no difference was observed between controls and PSVT patients ($P > 0.05$). The AUC was 0.755 (95%CI 0.671–0.838, $P < 0.001$) and at a cut-off point at

0.875, with 65.9% sensitivity and 75.6% specificity, the PPV was 83.6% and the NPV was 54.0% (Figure 3B). The OR values of GAS5 were 0.224 (95%CI 0.058–0.873, $P = 0.031$) between AF and PSVT patients (Table 4). That is to say, GAS5 had the ability to distinguish AF from different diseases with similar symptoms.

Table 4 Multivariate logistic analysis for the association of GAS5 with clinical characteristics between AF and PSVT patients.

	P value	Odds ratio	95% Confidence interval
Age(years)	0.145	0.936	0.856-1.023
Sex (female)	0.139	0.231	0.033-1.609
GAS5	0.031	0.224	0.058-0.873
Alcohol	0.008	0.062	0.008-0.479
BMI (kg/m ²)	0.021	1.295	1.040-1.612
Thyroid dysfunction	0.284	0.364	0.057-2.312
NT-proBNP (pg/ml)	0.226	1.007	0.996-1.018
LAVI (ml/m ²)	<0.001	1.269	1.137-1.415
Cr	0.036	1.079	1.005-1.159

Thyroid dysfunction means Subclinical or overt hyperthyroidism

PeAF expressed lower GAS5 level in plasma compared with PAF

AF patients were divided into paroxysmal and persistent based on different progression. Considering the imbalanced distribution of HT in these two groups (60.00% vs 37.14%), we divided all participants into HT+/- groups first. With or without HT, both PAF and PeAF had lower GAS5 level as compared to control. And in HT- patients, PeAF had lower expression of GAS5 compared with PAF ($P < 0.05$), whereas in HT+, no statistical significance was displayed (Figure 4A/B). These results identified that GAS5 further downregulated in PeAF patients who had more heavier AF burden.

Association between GAS5 and clinical characteristics in AF patients

Table 5 showed the association between GAS5 and clinical characteristics in AF patients demonstrating that GAS5 was negatively correlated with age, cystatin C and CHA2DS2-VASc score ($P < 0.05$). After adjusting the interference of age, no correlation was observed between GAS5 and CHA2DS2-VASc score ($P = 0.487$). Considering the influence of age and HT on atrial size, we adjusted these two variates and found roughly negative correlation between GAS5 and certain echocardiography indexes containing LAD ($P = 0.051$), LAV ($P = 0.044$) and LAVI ($P = 0.051$). Then, we divided AF patients into three groups on the basis of CHA2DS2-VASc score and found that the expression of GAS5 decreased gradually with the

increase of score, especially in patients whose score was ≥ 2 in male or ≥ 3 in female ($P = 0.022$) (Figure 5).

Table 5 Spearman's rank correlation analysis for the association of GAS5 with clinical characteristics

	Coefficient	<i>P</i>		Coefficient	<i>P</i>
AF risk factors					
Age	-0.246	0.023	Smoking	-0.021	0.846
Sex	-0.051	0.641	Alcohol	0.129	0.241
Hypertension	-0.141	0.200	BMI	-0.041	0.709
Diabetes	0.036	0.741	BSA	0.048	0.660
Cystatin C	-0.250	0.021	Cr	0.060	0.586
eGFR	0.013	0.906	TSH	0.129	0.241
Hyperuricemia	0.009	0.934			
Cardiac typical biomarkers					
cTNI	-0.182	0.096	HBDH	0.058	0.599
AST	0.078	0.477	CK	0.098	0.371
LDH	0.068	0.537	CKMB	0.185	0.090
NT-proBNP	-0.056	0.609			
Echocardiography information					
LAD [#]	-0.215	0.051	LVEF	-0.077	0.486
LAV [#]	-0.221	0.044	LVIDd	0.070	0.526
LAVI [#]	-0.215	0.051			
Stroke risk score					
CHA2DS2-VASc	-0.228	0.036	CHA2DS2-VASc ^{##}	-0.077	0.487

Abbreviations and acronyms were shown in Table 1.

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

^{##} The coefficient and p value of CHA2DS2-VASc score was analyzed after adjusting age.

Discussion

Nowadays, lncRNAs as novel biomarkers had been active in many fields including cardiovascular diseases (CVD)[13, 25, 26]. These studies have exposed the attractive potential of lncRNAs as biomarkers in clinical diagnosis and prognosis. In this study, four lncRNAs closely correlated with CVD were selected. The screen experiment uncovered the down-regulation of GAS5 in AF patients, but no difference was found in other three lncRNAs.

GAS5 was rich in growth arrested cells and functioned as a riborepressor of the glucocorticoid receptor (GR) for its glucocorticoid response element-like sequence, suppressed the transcriptional of the GR, thereby modulated cell growth and metabolism[11]. Previous studies had identified GAS5 as attractively novel biomarker for non-small cell lung cancer (NSCLC) patients, which derived from plasma or exosome

and recognized patients in early stage, even functioned as a post-operation monitor[27, 28]. What's more, GAS5 was regarded as a meaningful prognostic predictor of survival and progression in glioblastoma patients[29]. Meanwhile, some studies have confirmed the stability of GAS5 expression in plasma promoting its use in clinic[30, 31]. In cardiovascular diseases, study had reported that GAS5 plays as a promising biomarker in coronary heart disease for its satisfying sensitivity and specificity[13]. And in HT patients' plasma, researchers also found a distinct down-regulation of GAS5 expression[12]. A recent microarray analysis recommended GAS5 as a novel biomarker in atrial tissues which displayed strong diagnostic power for AF through constructing an AF-related lncRNA-mRNA network (AFLMN). Functional module analysis deeply revealed that GAS5 was closely associated with 52 mRNAs and enriched in many AF-associated pathways involved in inflammation, ion channel and metabolism regulation[32].

In this study, 50.59% AF patients experienced HT which was consistent with the data reported previously[33]. HT was known to be a major risk factor of AF. Recent research had found the down-regulation of GAS5 in HT patient and models' plasma indicating the role of GAS5 in HT progress. GAS5 was enriched in endothelial cell (EC) and vascular smooth muscle cell (VSMC) and down-regulated GAS5 in HT led to dysfunction of these two cells, finally caused arterial remodeling[12]. Considering the influence of HT in GAS5, we divided every groups into patients with HT or not and found a weak decrease of GAS5 expression without statistical significance which was not entirely consistent with the results in previous study[12]. This condition may attribute to shortage of sample size and better blood pressure control of subjects in our study. A prospective observational study exploring atrial electroanatomic remodeling from HT between PSVT and AF patients was proceeded in 2014. Researchers found some electroanatomic variations attributed to HT in PSVT patients promote the susceptibility and duration of AF, and more remarkable alter of electroanatomic was discovered in AF patients with HT. That is to say, HT distinctly facilitated the progress of AF[34]. Further comparison in our study between HT patients with or without AF proved a reduction of GAS5 in the former suggesting its specific effect in AF regardless of HT.

A total of 128 participants were enrolled ultimately including 85 AF patients and 43 controls. Result showed that GAS5 expression decreased in AF patients and the AUC of GAS5 was 0.858, with high level of sensitivity, specificity and positive predictive value. In addition, GAS5 was considered to be a protective factor for AF (OR = 0.039). These data showed its role as a suitable biomarker for AF patients. Additionally, we added patients who underwent PSVT recently as a special control group to extra examine the diagnosis power of GAS5. Paroxysmal supraventricular tachycardia is an innocuous arrhythmia which hardly cause cardiac remodeling but has similar symptoms to AF[35]. Both of them have to received rhythm or rate control if symptom intolerant, but their long-term therapies were widely divergent. A previous study reported that approximately 12% PSVT would suffer AF one year later showing their possible connections and evolution[36]. Another study followed up 1187 PSVT patients for 4.48 years in mean and found the prevalence of AF was 5%, lower than previous date but still higher than general population[37]. In view of these studies, PSVT patient was regarded as an ideal supplement to deeply evaluate the diagnostic value of GAS5 for its crossover with AF. Results demonstrated that the circulating

level of GAS5 remarkably decreased in AF patients compared with PSVT showing similar diagnostic value. And no difference was observed between PSVT and control.

As for AF patients, people were divided into two types according to different disease progressions. PAF experienced lower AF burden and higher success rate of conversion compared with PeAF. After 10 years' follow-up, more than 50% of PAF developed to persistent, but no statistic difference was observed between stroke and AF type[38]. Furthermore, PeAF was characterized by more serious electrical and structural remodeling which were primary pathophysiological variations for AF recurrence[39]. And Tao et al. proved that GAS5 suppress cardiac fibroblast activation and fibrosis by miR-21/PTEN /MMP-2 axis which were hallmark of AF structural remodeling, characterized by the down-regulation of Type I collagen (Col1A1) and Smooth muscle alpha-actin (α -SMA) expression[14, 15]. Recently, Liu et al. observed a markedly downregulation of GAS5 in mice model reduced by isoproterenol, which caused cardiac fibrosis and demonstrated that overexpressed GAS5 attenuates this pathological process[40]. That's to say, GAS5 played crucial role in cardiac fibrosis in vitro and vivo, suggesting its role in AF structural remodeling. Our studies confirmed a further downregulation of circulating GAS5 in PeAF patients with no history of HT ($P < 0.05$), demonstrating that the level of GAS5 may prognose the progress of AF. And the circulating level of GAS5 was negatively associated with LAD, LAV and LAVI, which reflected the volume of left atrium. Lower expression of GAS5 reflected heavier AF burden and larger atrial volume, while this aggravating burden itself could deteriorate atrial structural remodeling, partly reflected in the enlargement of atrial volume.

There are some limitations in our study. Firstly, the selection of lncRNA is partial. We expect that many other lncRNAs are regulated in AF and may act as biomarkers. Secondly, the number of samples in our study is small and monocentric. Large-scale and multicentric trials are needed to further verify the particular role of GAS5 as a biomarker. Thirdly, this is a retrospective study which lacks of prospective record, most of AF patients in our study went through radiofrequency ablation and long-term follow-up is under way.

Conclusion

In conclusion, we identified the role of GAS5 as a novel biomarker for AF diagnosis. Its downregulation was not observed in patients who had similar symptoms to AF but no cardiac remodeling. What's more, circulating level of GAS5 may prognose AF progression and stroke risk.

Abbreviations

GAS5: Growth arrest specific 5; HT: hypertension; PSVT: paroxysmal supraventricular tachycardia; AF: atrial fibrillation; PAF: paroxysmal atrial fibrillation; PeAF: persistent atrial fibrillation; BMI: body mass index; BSA: body surface area; SP: systolic pressure; DP: diastolic pressure; LAD: left atrium dimension; LVEF: left ventricular ejection fraction; LVIDd: left ventricular diastolic dimension; LAVI: left atrium volume index; LVPWd: left ventricular posterior wall diastolic dimension; IVSd: interventricular septum diastolic dimension; LVPWs: left ventricular posterior wall systolic dimension; LVM: left ventricular mass; LVMI: left

ventricular mass index; LVIDs: left ventricular systolic dimension; IVSs: interventricular septum systolic dimension; hsCRP: hypersensitive C-reactive protein; hsTnI: hypersensitive troponin I; NT-proBNP: N-terminal pro-B type natriuretic peptide; eGFR: estimated glomerular filtration rate; Cr: creatinine; TG: triglyceride; TC: total cholesterol; TSH: thyroid stimulating hormone; FBG: fasting blood glucose.

Declarations

Acknowledgments

We acknowledge all patients who agree to participate in this study.

Authors' contributions

XSH conceived this idea. JRS designed the study methodology. JRS, SC, BFW and KY conducted the study. JRS, SC, BFW analyzed the data. JRS, SC and KY interpreted the results. JRS and XSH wrote the draft manuscript. All authors took part in rewriting and approval of the final manuscript.

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Availability of data and materials

The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital, Medical School of Zhejiang University according to the Declaration of Helsinki. All subjects signed the informed consent to take part in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest with the contents of this article.

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References

- 1.Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, Gillum RF, Kim Y-H, McAnulty JH, Zheng Z-J *et al*: *Worldwide Epidemiology of Atrial Fibrillation A Global Burden of Disease 2010 Study. Circulation* 2014, *129*(8):837–847.
- 2.Kim MH, Johnston SS, Chu B-C, Dalal MR, Schulman KL: *Estimation of Total Incremental Health Care Costs in Patients With Atrial Fibrillation in the United States. Circulation-Cardiovascular Quality And Outcomes* 2011, *4*(3):313–320.
- 3.Miyasaka Y, Barnes ME, Gersh BJ, Cha SS, Bailey KR, Abhayaratna WP, Seward JB, Tsang TSM: *Secular trends in incidence of atrial fibrillation in Olmsted County, Minnesota, 1980 to 2000, and implications on the projections for future prevalence. Circulation* 2006, *114*(2):119–125.
- 4.Freedman B, Potpara TS, Lip GYH: *Stroke prevention in atrial fibrillation. Lancet* 2016, *388*(10046):806–817.
- 5.Kotecha D, Piccini JP: *Atrial fibrillation in heart failure: what should we do? European Heart Journal* 2015, *36*(46):3250-U3258.
- 6.Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, Castella M, Diener H-C, Heidbuchel H, Hendriks J *et al*: *2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. European Heart Journal* 2016, *37*(38):2893+.
- 7.Quinn JJ, Chang HY: *Unique features of long non-coding RNA biogenesis and function. Nature Reviews Genetics* 2016, *17*(1):47–62.
- 8.Uchida S, Dimmeler S: *Long Noncoding RNAs in Cardiovascular Diseases. Circulation Research* 2015, *116*(4):737–750.
- 9.Gurha P: *Noncoding RNAs in cardiovascular diseases. Current opinion in cardiology* 2019, *34*(3):241–245.
- 10.Viereck J, Thum T: *Circulating Noncoding RNAs as Biomarkers of Cardiovascular Disease and Injury. Circulation Research* 2017, *120*(2):381–399.

11. Kino T, Hurt DE, Ichijo T, Nader N, Chrousos GP: *Noncoding RNA Gas5 Is a Growth Arrest- and Starvation-Associated Repressor of the Glucocorticoid Receptor. Science Signaling* 2010, 3(107).
12. Wang Y-N-Z, Shan K, Yao M-D, Yao J, Wang J-J, Li X, Liu B, Zhang Y-Y, Ji Y, Jiang Q et al: *Long Noncoding RNA-GAS5 A Novel Regulator of Hypertension-Induced Vascular Remodeling. Hypertension* 2016, 68(3):736-+.
13. Yin Q, Wu A, Liu M: *Plasma Long Non-Coding RNA (lncRNA) GAS5 is a New Biomarker for Coronary Artery Disease. Medical Science Monitor* 2017, 23:6042-6048.
14. Tao H, Zhang J-G, Qin R-H, Dai C, Shi P, Yang J-J, Deng Z-Y, Shi K-H: *LncRNA GAS5 controls cardiac fibroblast activation and fibrosis by targeting miR-21 via PTEN/MMP-2 signaling pathway. Toxicology* 2017, 386:11-18.
15. Alessie M, Ausma J, Schotten U: *Electrical, contractile and structural remodeling during atrial fibrillation. Cardiovascular Research* 2002, 54(2):230-246.
16. Adriaens C, Standaert L, Barra J, Iatil M, Verfaillie A, Kalev P, Boeckx B, Wijnhoven PWG, Radaelli E, Vermi W et al: *p53 induces formation of NEAT1 lncRNA-containing paraspeckles that modulate replication stress response and chemosensitivity. Nature Medicine* 2016, 22(8):861-+.
17. Liu Z, Wu K, Wu J, Tian D, Chen Y, Yang Z, Wu A: *NEAT1 is a potential prognostic biomarker for patients with nasopharyngeal carcinoma. Journal Of Cellular Biochemistry* 2019, 120(6):9831-9838.
18. Ahmed ASI, Dong K, Liu J, Wen T, Yu L, Xu F, Kang X, Osman I, Hu G, Bunting KM et al: *Long noncoding RNA NEAT1 (nuclear paraspeckle assembly transcript 1) is critical for phenotypic switching of vascular smooth muscle cells. Proceedings Of the National Academy Of Sciences Of the United States Of America* 2018, 115(37):E8660-E8667.
19. Gast M, Rauch B, Haghikia A, Nakagawa S, Haas J, Stroux A, Schmidt D, Schumann P, Weiss S, Jensen L et al: *Long noncoding RNA NEAT1 modulates immune cell functions and is suppressed in early onset myocardial infarction patients. Cardiovascular research* 2019.
20. Zhu Y, Feng Z, Jian Z, Xiao Y: *Long noncoding RNA TUG1 promotes cardiac fibroblast transformation to myofibroblasts via miR-29c in chronic hypoxia. Molecular Medicine Reports* 2018, 18(3):3451-3460.
21. Shi L, Tian C, Sun L, Cao F, Meng Z: *The lncRNA TUG1/miR-145-5p/FGF10 regulates proliferation and migration in VSMCs of hypertension. Biochemical And Biophysical Research Communications* 2018, 501(3):688-695.
22. Li FP, Lin DQ, Gao LY: *LncRNA TUG1 promotes proliferation of vascular smooth muscle cell and atherosclerosis through regulating miRNA-21/PTEN axis. European Review for Medical And Pharmacological Sciences* 2018, 22(21):7439-7447.

23. Wang X-S, Zhang Z, Wang H-C, Cai J-L, Xu Q-W, Li M-Q, Chen Y-C, Qian X-P, Lu T-J, Yu L-Z *et al*: *Rapid identification of UCA1 as a very sensitive and specific unique marker for human bladder carcinoma. Clinical Cancer Research* 2006, *12*(16):4851–4858.
24. Liu Y, Zhou D, Li G, Ming X, Tu YF, Tian J, Lu H, Yu B: *Long Non Coding RNA-UCA1 Contributes to Cardiomyocyte Apoptosis by Suppression of p27 Expression. Cellular Physiology And Biochemistry* 2015, *35*(5):1986–1998.
25. Yan Y, Zhang B, Liu N, Qi C, Xiao Y, Tian X, Li T, Liu B: *Circulating Long Noncoding RNA UCA1 as a Novel Biomarker of Acute Myocardial Infarction. Biomed Research International* 2016.
26. Xuan L, Sun L, Zhang Y, Huang Y, Hou Y, Li Q, Guo Y, Feng B, Cui L, Wang X *et al*: *Circulating long non-coding RNAs NRON and MHRT as novel predictive biomarkers of heart failure. Journal Of Cellular And Molecular Medicine* 2017, *21*(9):1803–1814.
27. Tan Q, Zuo J, Qiu S, Yu Y, Zhou H, Li N, Wang H, Liang C, Yu M, Tu J: *Identification of circulating long non-coding RNA GAS5 as a potential biomarker for non-small cell lung cancer diagnosis. International Journal Of Oncology* 2017, *50*(5):1729–1738.
28. Li C, Lv Y, Shao C, Chen C, Zhang T, Wei Y, Fan H, Lv T, Liu H, Song Y: *Tumor-derived exosomal lncRNA GAS5 as a biomarker for early-stage non-small-cell lung cancer diagnosis. Journal of cellular physiology* 2019.
29. Shen J, Hodges TR, Song R, Gong Y, Calin GA, Heimberger AB, Zhao H: *Serum HOTAIR and GAS5 levels as predictors of survival in patients with glioblastoma. Molecular Carcinogenesis* 2018, *57*(1):137–141.
30. Han L, Ma P, Liu S-M, Zhou X: *Circulating long noncoding RNA GAS5 as a potential biomarker in breast cancer for assessing the surgical effects. Tumor Biology* 2016, *37*(5):6847–6854.
31. Liang W, Lv T, Shi X, Liu H, Zhu Q, Zeng J, Yang W, Yin J, Song Y: *Circulating long noncoding RNA GAS5 is a novel biomarker for the diagnosis of nonsmall cell lung cancer. Medicine* 2016, *95*(37).
32. Qian C, Li H, Chang D, Wei B, Wang Y: *Identification of functional lncRNAs in atrial fibrillation by integrative analysis of the lncRNA-mRNA network based on competing endogenous RNAs hypothesis. Journal Of Cellular Physiology* 2019, *234*(7):11620–11630.
33. Healey JS, Connolly SJ: *Atrial fibrillation: Hypertension as a causative agent, risk factor for complications, and potential therapeutic target. American Journal Of Cardiology* 2003, *91*(10):9G–14G.
34. Yin X, Zhao Y, Xi Y, Cheng N, Xia Y, Zhang S, Dong Y, Chang D, Cheng J, Yang Y *et al*: *The Early Stage of the Atrial Electroanatomic Remodeling as Substrates for Atrial Fibrillation in Hypertensive Patients. Journal Of the American Heart Association* 2014, *3*(5).

35. Page RL, Wilkinson WE, Clair WK, McCarthy EA, Pritchett ELC: *ASYMPTOMATIC ARRHYTHMIAS IN PATIENTS WITH SYMPTOMATIC PAROXYSMAL ATRIAL-FIBRILLATION AND PAROXYSMAL SUPRAVENTRICULAR TACHYCARDIA*. *Circulation* 1994, 89(1):224–227.
36. Hamer ME, Wilkinson WE, Clair WK, Page RL, McCarthy EA, Pritchett ELC: *INCIDENCE OF SYMPTOMATIC ATRIAL-FIBRILLATION IN PATIENTS WITH PAROXYSMAL SUPRAVENTRICULAR TACHYCARDIA*. *Journal Of the American College Of Cardiology* 1995, 25(5):984–988.
37. Khachab H, Brembilla-Perrot B: *Prevalence of atrial fibrillation in patients with history of paroxysmal supraventricular tachycardia*. *International Journal Of Cardiology* 2013, 166(1):221–224.
38. Padfield GJ, Steinberg C, Swampillai J, Qian H, Connolly SJ, Dorian P, Green MS, Humphries KH, Klein GJ, Sheldon R *et al*: *Progression of paroxysmal to persistent atrial fibrillation: 10-year follow-up in the Canadian Registry of Atrial Fibrillation*. *Heart Rhythm* 2017, 14(6):801–807.
39. Dzeshka MS, Lip GYH, Snezhitskiy V, Shantsila E: *Cardiac Fibrosis in Patients With Atrial Fibrillation Mechanisms and Clinical Implications*. *Journal Of the American College Of Cardiology* 2015, 66(8):943–959.
40. Liu HL, Chen CH, Sun YJ: *Overexpression of lncRNA GAS5 attenuates cardiac fibrosis through regulating PTEN/MMP–2 signal pathway in mice*. *European Review for Medical And Pharmacological Sciences* 2019, 23(10):4414–4418.

Figures

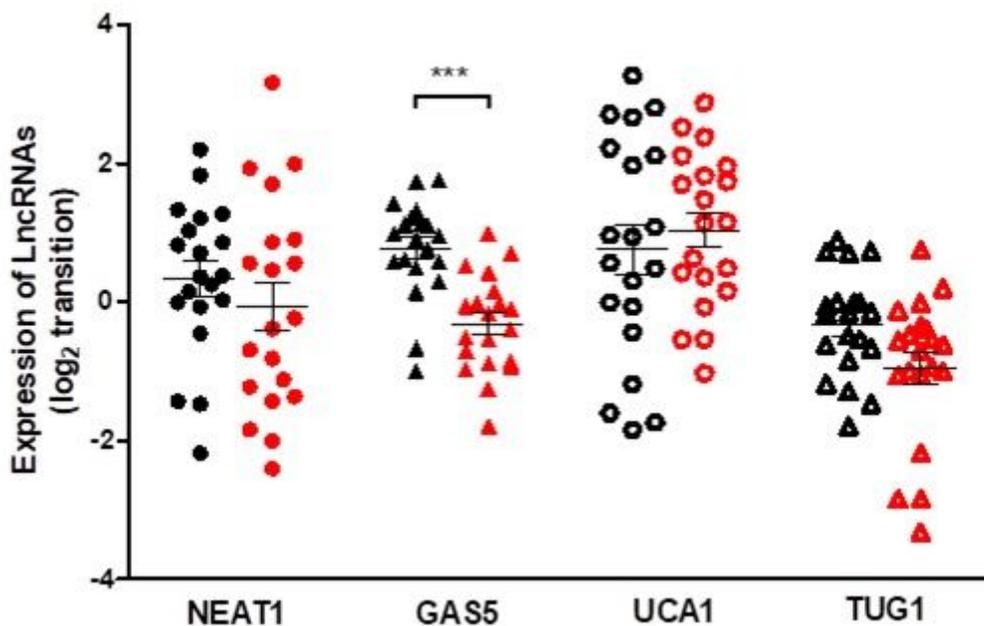


Figure 1

Validation of four candidate lncRNAs identified in screening study between AF patients and controls. The expression levels of four candidate lncRNAs in 20 AF patients and 20 healthy controls were analyzed by qRT-PCR. *** means $P < 0.001$

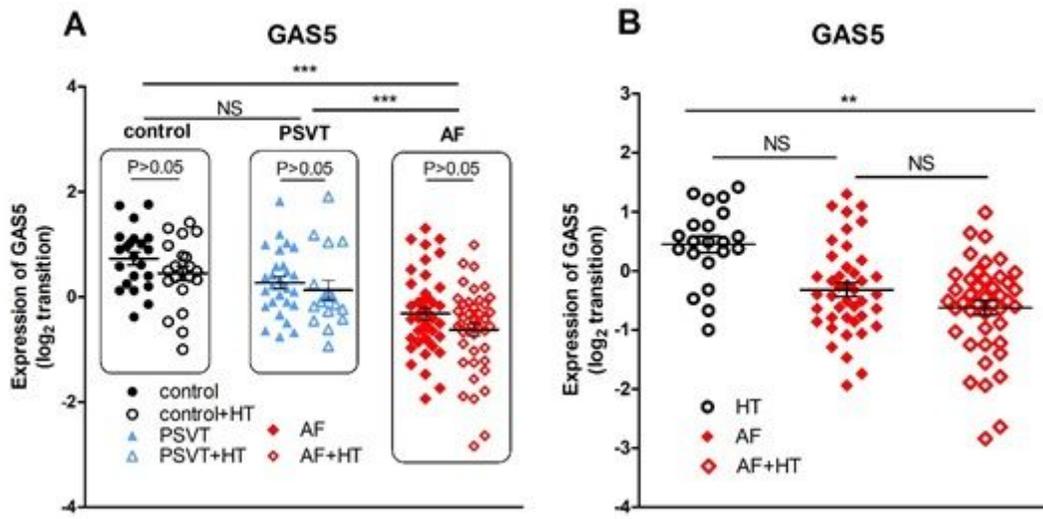


Figure 2

Relative expression of GAS5 in controls, AF and PSVT with or without hypertension. (A) A total of 128 subjects enrolled in study including 85 AF patients and 43 controls. 45 PSVT patients were selected as specific controls to further evaluate the diagnostic value. Compared with controls, AF patients expressed lower level of GAS5. The same trend was observed between PSVT and AF patients. And no statistical significance was shown between PSVT and control. All groups were divided into two subgroups according to whether they had hypertension or not and no significance was shown. (B) Compared with HT patients in controls, circulating GAS5 further decreased in HT patients with AF ** $P < 0.01$; *** $P < 0.001$;

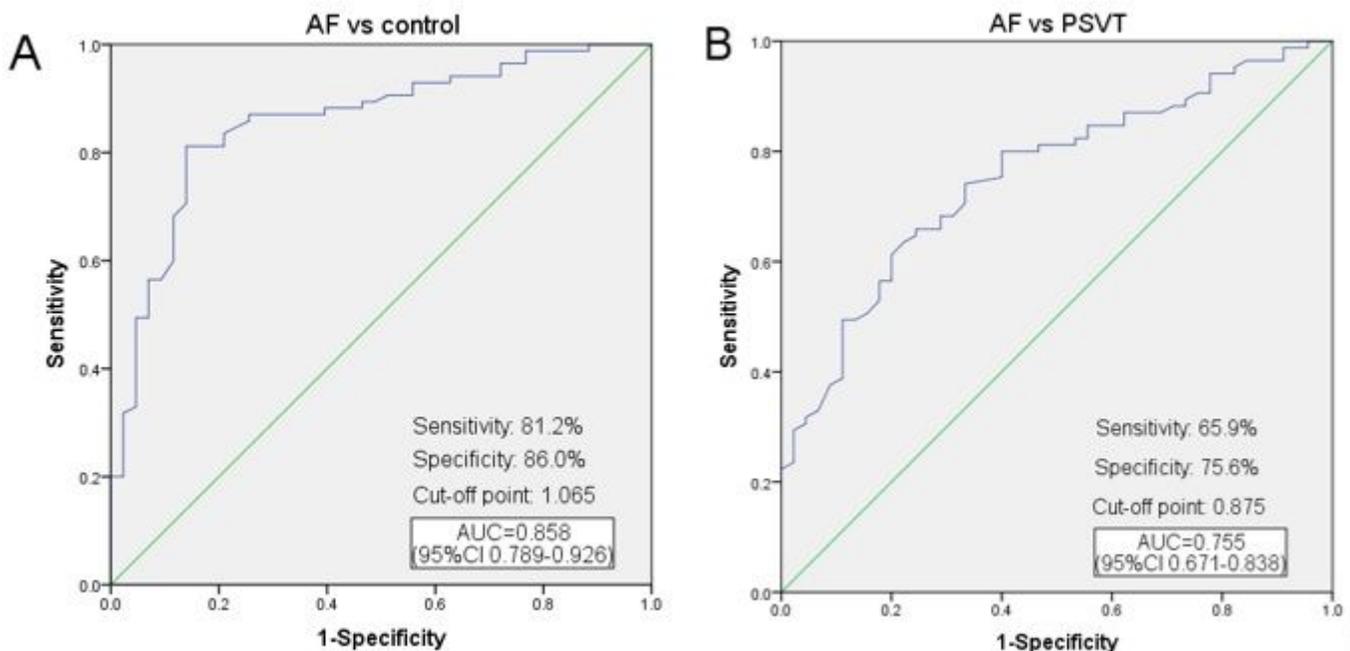


Figure 3

Receiver operating characteristic curve (ROC) analysis of GAS5 for AF diagnosis. (A) the diagnostic value of GAS5 in AF patients compared with controls. (B) the diagnostic value of GAS5 in AF patients compared with PSVT patients.

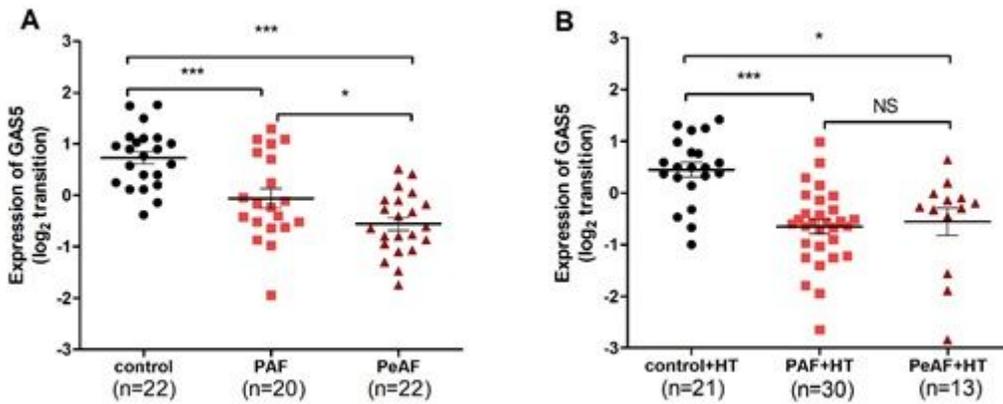


Figure 4

The expression of GAS5 in PAF and PeAF patients with or without HT. (A) In HT- patients, PAF had a higher expression of GAS5 compared with PeAF. The level of GAS5 expressed in PAF or PeAF patients were lower than controls, respectively. (B) In HT+ patients, no statistical significance was displayed between PAF and PeAF. Compared with controls, the expression level of GAS5 in PAF or PeAF patients evidently decreased. * P<0.05; *** P<0.001.

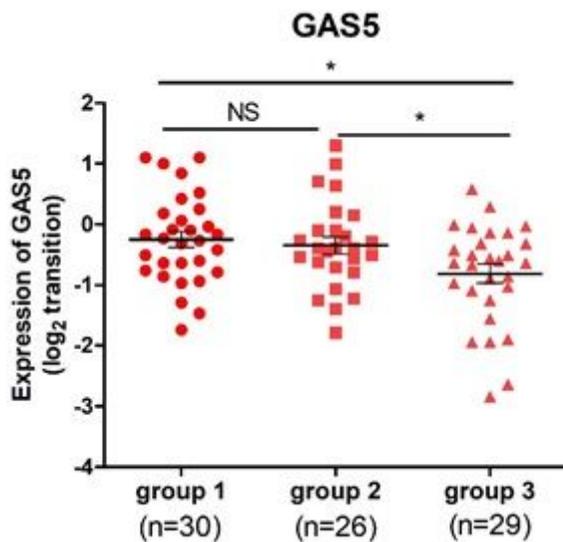


Figure 5

The expression of GAS5 in AF patients with different CHA₂DS₂-VASc score. Group 1 represented AF patients whose score was 0 in male or 1 in female; Group 2 represented AF patients whose score was 1 in male or 2 in female; Group 3 represented AF patients whose score was ≥2 in male or ≥3 in female. Patients in groups had convictive decrease compared with group 1 or group 2 respectively

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