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ORIGINAL ARTICLE

Serum relaxin level predicts recurrence of atrial fibrillation after radiofrequency catheter ablation

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Abstract

Relaxin, an emerging biomarker in heart failure, is involved in fibrosis and inflammation. The value of relaxin in predicting recurrence of atrial fibrillation (AF) after radiofrequency catheter ablation (RFCA) is unknown and the subject of this study. We prospectively enrolled 248 consecutive patients with AF (paroxysmal in 127 and persistent in 121) who underwent RFCA at our center after measurement of circulating levels of relaxin by ELISA. Kaplan–Meier analysis with log-rank test and multivariate analysis were used to assess the association between pre-RFCA relaxin levels and post-RFCA AF recurrence at 18 months follow-up. At mean 16.3 ± 3.8 months post-RFCA, 195 (78.6%) patients maintained sinus rhythm, and their pre-RFCA relaxin level was lower than that in patients with AF recurrence ($P < 0.001$). From lowest to highest pre-RFCA relaxin level tertiles (T1; 82.10–< 234.36; T2; 234.36–< 342.26; and T3; 342.26–740.63 ng/L), AF recurrence rate increased significantly (8.5%, 20.5% and 34.9%, respectively; Kaplan–Meier analysis with log-rank test, $\chi^2 = 18.44$, $P < 0.001$). Using a cutoff of 285.4 ng/L, pre-RFCA relaxin level predicted AF recurrence during follow-up with sensitivity of 77.4% and specificity of 55.9% (area under the receiver operating characteristic curve = 0.71). On multivariate Cox proportional hazard model, relaxin level by tertile (T2, hazard ratio 2.678; 95% confidence interval 1.110–6.460; $P = 0.028$, and T3, hazard ratio 4.745; 95% confidence interval 2.075–10.854; $P < 0.001$, respectively compared with the T1) was the independent factor predicting recurrence. Elevated pre-RFCA relaxin level is associated with post-RFCA AF recurrence. A simple measurement of relaxin level therefore might help identify patients at high risk of AF recurrence after RFCA.
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Keywords Atrial fibrillation · Ablation · Recurrence · Relaxin

Abbreviations

AF	Atrial fibrillation
RFCA	Radiofrequency catheter ablation
Ang II	Renin–angiotensin–aldosterone system and angiotensin II
PaAF	Paroxysmal atrial fibrillation
PeAF	Persistent atrial fibrillation
ECG	Electrocardiography
eGFR	Estimated glomerular filtration rate
BNP	Brain natriuretic peptide
LAD	Left atrial diameter
LVMI	Left ventricular mass index
ROC	Receiver operating characteristic
CI	Confidence interval
AUC	Area under the ROC curve
HR	Hazard ratio
OR	Odds ratio
ECM	Extracellular matrix
LA	Left atrial
LDL-C	Low-density lipoprotein cholesterol
LVEDD	Left ventricular end-diastolic diameter

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Introduction

Electrophysiological and structural abnormalities promote abnormal impulse formation and propagation leading to genesis, progression and recurrence of atrial fibrillation (AF), the most common cardiac arrhythmia with substantial morbidity and mortality [1–3]. Symptomatic and drug-refractory AF are commonly treated with radiofrequency catheter ablation (RFCA) [4, 5]; however, the rates of AF recurrence after RFCA are 25% to 50% [4] and even higher for non-paroxysmal AF [6]. Atrial fibrosis and atrial structural remodeling are key substrates for AF recurrence post-RFCA [7–9], particularly of persistent AF.

Identification of easily measured pre-RFCA predictors of post-RFCA AF recurrence would inform optimization and research of treatment strategies. Various biochemical markers have yielded inconsistent results as predictors of post-RFCA AF [10]. Relaxin, as a naturally occurring human hormone, down-regulated the deposition of collagen and other extracellular matrix proteins, reduced oxidative stress and diminished inflammatory response. A number of downstream pathological processes are involved, including prevention or reversal of the effects of the neurohormones Ang II, reduced expression of transforming growth factor β and increased activation of matrix metalloproteinases, which results in reduced fibrosis [11]. Several animal experiments have demonstrated that relaxin could attenuate both the proliferation of cardiac fibroblasts and the synthesis of collagen in cardiac fibroblast cells [12].

Given that atrial fibrosis is a common feature of clinical AF and relaxin can inhibit fibroblast activation and collagen synthesis in cardiac diseases, we speculated that relaxin might be a useful marker for risk stratification in the management of AF. Therefore, this study assessed the value of serum relaxin level pre-RFCA as predictor of AF recurrence post-RFCA.

Methods

Study population

This prospective and observational study enrolled 248 consecutive AF patients undergoing left atrial RFCA at the First affiliated Hospital of Wenzhou Medical University. The study protocol was approved by the Ethics Review Board of the First Affiliated Hospital of Wenzhou Medical University, and all study participants provided written informed consent. The approval reference number is Clinical Research Ethics Approval (2013) number 38.

Patients were categorized by AF type, i.e., paroxysmal AF (PaAF) or persistent AF (PeAF), based on previous medical documentation and electrocardiography (ECG), dynamic ECG or cardiac telemetry system results during hospitalization [13]. AF was defined as cardiac arrhythmia with "absolutely" irregular RR intervals and no distinct P waves on surface ECG. PaAF was defined as AF that was self-terminating within 7 days after onset and documented by previous routine or dynamic ECG. PeAF was defined as any AF episode lasting longer than 7 days or requiring drug or direct current cardioversion for termination. AF's ECG was blindly adjudicated by a validation committee which referred to the Guidelines for the Management of Atrial Fibrillation (ESC) [14]. Key exclusion criteria, chosen based on their possibly affecting relaxin level, were: heart failure with New York Heart Association level III–IV, acute or previous myocardial infarction, history of previous RFCA because of AF, valvular AF and patients with renal or hepatic impairment. Renal impairment was defined as the presence of chronic dialysis or renal transplantation or serum creatinine ≥ 200 mmol/L. Hepatic impairment was defined as chronic hepatic disease (e.g., cirrhosis) or biochemical evidence of significant hepatic derangement (e.g., bilirubin more than two times upper limit of normal, in association with aspartate aminotransferase/alanine aminotransferase/alkaline phosphatase more than three times upper limit normal, etc.).

RFCA

Patients underwent transthoracic and transesophageal echocardiography before RFCA to confirm the absence of left atrial thrombus. Under deep propofol sedation by continuous intravenous micro-pump infusion throughout the entire RFCA procedure, patients were monitored for arterial blood pressure, oxygen saturation, and ECG. Via venous access and double trans-septal puncture, an open irrigated ablation catheter and a circular mapping catheter were positioned in the left atrium. A 3D mapping system was used to reconstruct left atrial geometry and for electroanatomic guidance. Circumferential pulmonary vein isolation was performed in PaAF cases. In patients with PeAF, additional linear lesions were added at the left atrial roof, basal posterior wall, and left atrial isthmus. The isolation of all pulmonary veins with bidirectional block was defined as the procedural end point and verified by using a multipolar circular mapping catheter.

After RFCA, patients remained under continuous blood pressure and ECG monitoring for 24 h. Class I and III antiarrhythmic drugs were not reinitiated. According to current guidelines [15], oral anticoagulation therapy was recommended peri-ablation.

Follow-up

After hospital discharge, all patients were followed up at the outpatient clinic for 18 months to identify the recurrence of AF via monthly 12-lead ECG and quarterly 24-h Holter recordings. After a blanking period of 3 months, AF recurrence was defined as any episode of symptomatic or asymptomatic AF or any atrial flutter seen on ECG or Holter recording that lasted > 30 s [4]. AF recurrence during follow-up was censored.

Related baseline and blood samples

At baseline, patients completed a standardized questionnaire to assess risk factors, including age, sex, duration of AF, smoking, alcohol intake, hypertension and diabetes. Physical examination included assessment of blood pressure, heart rate and body mass index. Laboratory measurements included estimated glomerular filtration rate (eGFR), hepatic transaminases, low-density lipoprotein cholesterol, fasting blood glucose and brain natriuretic peptide (BNP), with log base-e transformation for BNP (lnBNP) because it did not exhibit a normal distribution. Echocardiography measurements included left ventricular ejection fraction, left atrial diameter (LAD) by M-mode echocardiography in anteroposterior left atrial dimension of parasternal, left ventricular end-diastolic diameter, pulmonary arterial pressure cardiac output and left ventricular mass index (LVMI). Baseline peripheral vein blood samples were collected on the morning following hospitalization from patients in stable condition before ablation. Serum was separated at 2000 rpm for 20 min and stored at − 80 °C in plastic freezer vials. All the biochemical measurements were performed at the hospital's laboratory.

Determination of serum relaxin

Serum relaxin was measured by use of a commercial ELISA kit (Boyun, Shanghai, China) with a sensitivity (lower detection limit) of 10 ng/L. The polyclonal antibody was raised in rabbits. The intra-assay coefficient of variation was less than 14%, as well as the inter-assay coefficient of variation. The samples were run in duplicate and measured at 450λ.

Statistical analysis

SPSS version 20.0 (SPSS, Inc. Chicago, IL) was used for all data analyses. Continuous variables are expressed as mean ± SD and categorical variables as number (%). Continuous variables were compared by *t* test and categorical variables by Chi-square or Fisher's exact test. The Youden index (sensitivity + specificity − 1) of the receiver operating characteristic (ROC) curve was calculated to determine the best cutoff value for serum relaxin level to predict AF recurrence. Kaplan–Meier survival analysis was [performed with log-rank test. Univariate and multivariate analyses were performed using a Cox proportional hazard model to predict the AF recurrence after RFCA. Multivariate Cox proportional hazard model included variables with $P < 0.10$ on univariate analysis. Two-sided $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 248 patients (age range 31–87 years; 127 with PaAF and 121 with PeAF) who met the inclusion criteria were enrolled. After a mean of 16.3 ± 3.8 months follow-up, 53 patients (21.4%) had AF recurrence after RFCA, without significant difference between PaAF and PeAF patients for AF recurrence (16.5% and 26.4%, respectively; $P = 0.057$) as well as other parameters except eGFR ($P = 0.020$) (Table 1). Patients with and without AF recurrence had similar distribution of most characteristics (Table 2) except for relaxin level ($P < 0.001$) and LAD ($P = 0.018$).

Serum relaxin levels and predictive values for AF recurrence

Baseline serum relaxin levels were higher in patients with than without AF recurrence (382.21 ± 149.89 vs. 275.42 ± 108.70 ng/L, $P < 0.001$) (Table 2). We divided the 248 patients into tertiles based on pre-RFCA serum relaxin level: first tertile [T1; 82.10–< 234.36 ng/L, $n = 82$], second tertile [T2; 234.36–< 342.26 ng/L, $n = 83$] and third tertile [T3; 342.26–740.63 ng/L, $n = 83$]. The mean relaxin level in the 3 groups was 166.81 ± 33.88, 289.37 ± 31.25, and 436.96 ± 95.17 ng/mL, respectively. AF recurrence rates from the lowest to the highest relaxin tertiles were 8.5% ($n = 7$), 20.5% ($n = 17$) and 34.9% ($n = 29$), respectively ($P < 0.001$). AF recurrence rate significantly differed among the 3 tertiles of relaxin level by Kaplan–Meier analysis (log-rank test, $\chi^2 = 18.44$, $P < 0.001$) (Fig. 1). On post hoc analysis, the difference in AF recurrence rate between T1 and T3 patients was significant ($\chi^2 = 18.15$, $P < 0.001$).

On ROC curve analysis, a cutoff level of 285.4 ng/L for serum relaxin level predicted AF recurrence during follow-up with sensitivity of 77.4% (95% CI 63.8–87.7%) and specificity of 55.9% (95% CI 48.6–63.0%); the area under the ROC curve (AUC) was 0.71 (95% CI 0.63–0.79; $P < 0.001$) (Fig. 2). Risk of AF recurrence after RFCA was significantly higher with pre-RFCA serum relaxin level > 285.4 ng/mL (HR 4.33, 95% CI 2.15–8.74; $P < 0.001$).

Table 1 Baseline clinical characteristics of AF patients

Clinical parameters	Paroxysmal AF ($n=127$)	Persistent AF ($n=121$)	P value
Demographics			
Age (years)	64.50 ± 11.61	66.93 ± 9.82	0.077
Male sex	83 (65.4)	74 (61.2)	0.493
Body mass index (kg/m^2)	24.28 ± 3.28	24.61 ± 3.34	0.429
Heart rate (bpm)	78.56 ± 18.00	81.86 ± 15.71	0.126
Systolic blood pressure (mmHg)	133.43 ± 21.92	130.90 ± 19.78	0.341
Duration of AF (years)	2.98 ± 4.46	3.17 ± 4.09	0.739
Laboratory measurements			
Alanine aminotransferase (U/L)	26.30 ± 18.81	27.94 ± 19.85	0.505
Aspartate aminotransferase (U/L)	24.73 ± 9.45	26.66 ± 9.72	0.115
eGFR (mL/min/1.73 m^2)	112.07 ± 31.17	103.68 ± 24.82	0.020*
LDL-C level (mmol/L)	2.57 ± 0.97	2.42 ± 0.78	0.198
Serum glucose (mmol/L)	5.70 ± 5.47	5.30 ± 1.38	0.446
LnBNP level	4.46 ± 1.13	4.49 ± 1.20	0.856
Relaxin (ng/L)	293.51 ± 124.06	303.21 ± 128.80	0.546
Medical history			
Diabetes mellitus	20 (15.7)	22 (18.2)	0.609
Hypertension	64 (50.4)	70 (57.9)	0.239
Smoking	26 (20.5)	28 (23.1)	0.611
Alcohol use	20 (15.7)	26 (21.5)	0.245
AF recurrence	21 (16.5)	32 (26.4)	0.057

Data are mean ± SD or number (%)

AF atrial fibrillation, *eGFR* estimated glomerular filtration rate, *LDL-C* low-density lipoprotein cholesterol, *BNP* brain natriuretic peptide

*$P<0.05$

The high-risk factors of RFCA

We performed univariate and multivariate Cox proportional hazard model to analyze the independent factors associated with AF recurrence. Demographic data, vital signs, medical history, laboratory measurements, echocardiography data and relaxin level by tertile were used to predict AF recurrence after RFCA (Table 3). In multivariate analysis, relaxin level by tertile (T2, HR 2.678; 95% CI 1.110–6.460; $P=0.028$, and T3, HR 4.745; 95% CI 2.075–10.854; $P<0.001$, respectively, compared with the T1) was the independent factor predicting recurrence in this study.

Discussion

To the best of our knowledge, this study is the first to assess the association between pre-RFCA serum relaxin level and AF recurrence after RFCA. Relaxin level was greater for patients with than without AF recurrence, and on multivariate analysis, pre-RFCA serum relaxin level was associated with AF recurrence after RFCA. Patients in the highest tertile (T3) of relaxin level were the independent factor predicting recurrence.

AF and atrial fibrosis

Despite intensive research, the mechanisms underlying AF, the most prevalent rhythm disorder in clinical practice [16] and a major cause of morbidity and mortality [17], remain incompletely understood. Electrical, contractile, and structural remodeling has been associated with AF pathogenesis. Atrial fibrosis is the hallmark of structural remodeling [18, 19], which in the atrium of patients with AF has been identified at the level of cardiomyocytes and extracellular matrix (ECM) [20]. ECM remodeling, including ECM volume and composition, are untoward changes in cardiac structure and function during AF progression [21]. The latter findings underscore the association between atrial fibrosis and AF, although the mechanism of atrial fibrosis in AF occurrence and persistence is still not fully elucidated. This study reproduced the finding in other studies [22–24] of large atrial dimension as a result of atrial fibrosis being a risk factor and predictor of AF recurrence. Reversal of atrial fibrosis appears promising as a new treatment for AF and animal studies have documented its feasibility [25, 26]. Therefore, use of relaxin as an anti-fibrosis agent in AF patients might be possible as suggested by its beneficial effect on heart failure via ventricular fibrosis relief [27].

Table 2 Baseline clinical characteristics of study patients

Clinical parameters	Overall ($n=248$)	AF recurrence		P value
		No ($n=195$)	Yes ($n=53$)	
Demographics				
Age (years)	65.69 ± 10.82	65.64 ± 11.21	65.87 ± 9.32	0.893
Male sex	157 (63.3)	124 (63.6)	33 (62.3)	0.859
Body mass index (kg/m^2)	24.44 ± 3.31	24.28 ± 3.24	25.05 ± 3.51	0.132
Heart rate (bpm)	80.17 ± 16.97	79.49 ± 17.06	82.68 ± 16.58	0.225
Systolic blood pressure (mmHg)	132.20 ± 20.90	132.56 ± 20.70	130.85 ± 21.77	0.597
Diastolic blood pressure (mmHg)	77.81 ± 13.07	77.64 ± 13.14	78.43 ± 12.90	0.696
Duration of AF (years)	3.07 ± 4.28	3.12 ± 4.41	2.89 ± 3.81	0.727
Laboratory measurements				
Alanine aminotransferase (U/L)	27.11 ± 19.30	26.26 ± 18.36	30.19 ± 22.34	0.190
Aspartate aminotransferase (U/L)	25.68 ± 9.61	25.32 ± 9.30	26.98 ± 10.68	0.266
eGFR (mL/min/1.73 m^2)	107.98 ± 28.51	109.32 ± 29.50	103.01 ± 24.11	0.153
LDL-C level (mmol/L)	2.50 ± 0.88	2.53 ± 0.92	2.39 ± 0.74	0.322
Serum glucose (mmol/L)	5.51 ± 4.03	5.60 ± 4.51	5.18 ± 1.22	0.512
LnBNP level	4.48 ± 1.16	4.45 ± 1.15	4.58 ± 1.20	0.455
Relaxin level (ng/L)	298.24 ± 126.23	275.42 ± 108.70	382.21 ± 149.89	< 0.001*
Medical history				
Diabetes mellitus	42 (16.9)	34 (17.4)	8 (15.1)	0.687
Hypertension	134 (54.0)	106 (54.4)	28 (52.8)	0.843
Smoking	54 (21.8)	39 (20.0)	15 (28.3)	0.194
Alcohol use	46 (18.5)	33 (16.9)	13 (24.5)	0.207
Echocardiography				
Left ventricular ejection fraction (%)	62.10 ± 9.62	62.50 ± 9.64	60.7 ± 9.5	0.221
LAD (mm)	45.76 ± 5.79	45.30 ± 5.87	47.42 ± 5.23	0.018*
LVEDD (mm)	50.27 ± 5.54	50.22 ± 5.52	50.45 ± 5.65	0.791
Pulmonary arterial pressure (mmHg)	34.26 ± 9.27	33.80 ± 9.14	35.92 ± 9.63	0.140
Cardiac output (L/min)	5.48 ± 1.70	5.47 ± 1.69	5.53 ± 1.76	0.832
LVMI (g/m^2)	104.90 ± 27.77	105.06 ± 27.73	104.31 ± 28.16	0.862

Data are mean ± SD or number (%)

AF atrial fibrillation, *eGFR* estimated glomerular filtration rate, *LDL-C* low-density lipoprotein cholesterol, *BNP* brain natriuretic peptide, *LAD* left atrial diameter, *LVEDD* left ventricular end-diastolic dimension, *LVMI* left ventricular mass index

*$P < 0.05$

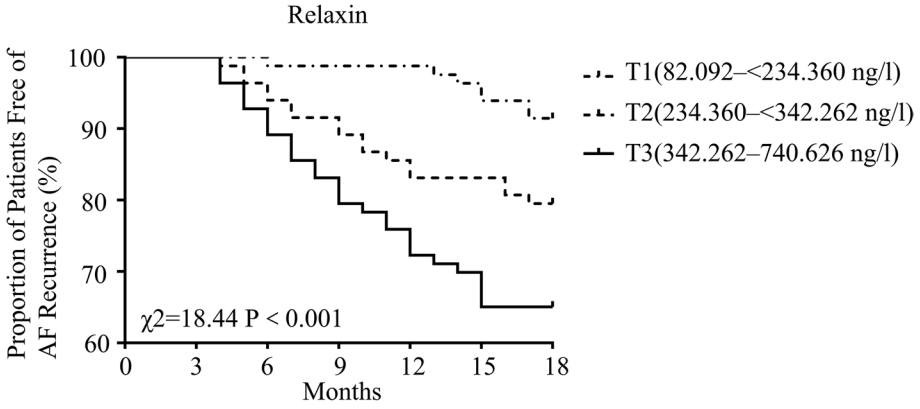

Fig. 1 Kaplan–Meier curve analysis of AF recurrence. Proportion of patients free from atrial fibrillation recurrence after radiofrequency catheter ablation by tertile of serum relaxin level (log-rank test, $\chi^2 = 18.44$, $P < 0.001$)

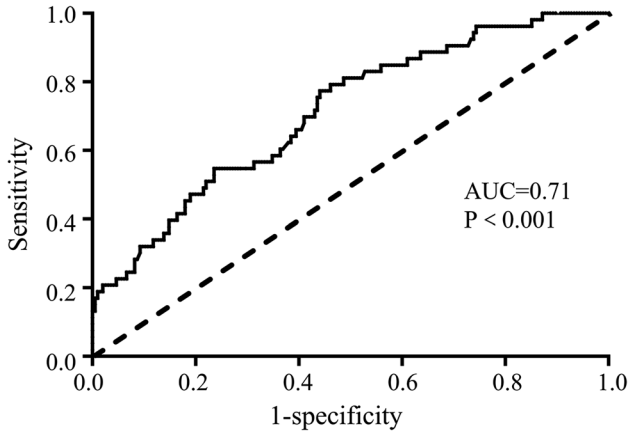

Fig. 2 Receiver operator characteristic (ROC) curve analysis of relaxin level for predicting atrial fibrillation recurrence after radiofrequency catheter ablation (area under the ROC curve = 0.71)

Relaxin in AF

Relaxin is a naturally occurring human peptide initially identified as a reproductive hormone. More recently, relaxin was found to play a key role in cardiovascular hemodynamics [28]. Relaxin has pleiotropic effects, including anti-inflammatory, ECM remodeling, Ang II-inhibiting, potent antifibrotic, and other cardioprotective activities [27], which also would be beneficial in AF management. According to numerous studies, attenuation of atrial fibrosis is a plausible approach to AF treatment [3, 12, 29]. In addition, relaxin increases conduction velocity by a combination of reversing fibrosis and hypertrophy and increasing fast sodium current in spontaneously hypertensive rat hearts to suppress AF [30].

Delayed-enhancement magnetic resonance imaging as a semi-quantitative method of quantification has been used to

Table 3 Independent factors for predicting AF recurrence after radiofrequency catheter ablatio patients

	Univariate			Multivariate		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Age (years)	1.001	0.977–1.026	0.919			
Sex, male, *n* (%)	0.982	0.744–1.296	0.896			
Body mass index (kg/m^2)	1.057	0.977–1.144	0.167			
Heart rate (bpm)	1.009	0.994–1.025	0.231			
Systolic blood pressure (mmHg)	0.996	0.983–1.009	0.551			
Diastolic blood pressure (mmHg)	1.004	0.983–1.025	0.713			
Duration of AF (years)	0.987	0.924–1.054	0.695			
Alanine aminotransferase (U/L)	1.008	0.996–1.020	0.197			
Aspartate aminotransferase (U/L)	1.016	0.990–1.043	0.229			
eGFR (mL/min/1.73 m^2)	0.993	0.984–1.002	0.143			
LDL-C level (mmol/L)	0.857	0.626–1.171	0.332			
Serum glucose (mmol/L)	0.962	0.852–1.086	0.528			
LnBNP level	1.097	0.862–1.397	0.450			
Relaxin level by tertile						
T1		1			1	
T2	2.631	1.091–6.344	0.031*	2.678	1.110–6.460	0.028*
T3	4.954	2.169–11.316	< 0.001*	4.745	2.075–10.854	< 0.001*
Diabetes mellitus, *n* (%)	1.100	0.755–1.602	0.620			
Hypertension, *n* (%)	1.035	0.790–1.356	0.802			
Smoking, *n* (%)	0.838	0.622–1.130	0.247			
Alcoholism, *n* (%)	0.844	0.617–1.154	0.287			
Left ventricular ejection fraction (%)	0.984	0.959–1.009	0.211			
LAD (mm)	1.050	1.006–1.096	0.026*	1.043	1.000–1.089	0.053
LVEDD (mm)	1.005	0.958–1.055	0.828			
Pulmonary arterial pressure (mmHg)	1.018	0.993–1.043	0.152			
Cardiac output (L/min)	1.022	0.870–1.200	0.793			
LVMI (g/m^2)	0.999	0.989–1.009	0.818			

AF atrial fibrillation, *HR* hazard ratio, *CI* confidence interval, *eGFR* estimated glomerular filtration rate, *LDL-C* low-density lipoprotein cholesterol, *BNP* brain natriuretic peptide, *LAD* left atrial diameter, *LVEDD* left ventricular end-diastolic dimension, *LVMI* left ventricular mass index

*$P < 0.05$

evaluate atrial fibrosis; however, quantifying atrial fibrosis in AF remains limited [31]. In our previous study, we found significantly higher levels of serum relaxin in patients with AF as compared with those in sinus rhythm, and the level could be used to quantify the extent of atrial fibrosis [13]. In the present study, relaxin levels pre-RFCA were similar between patients with PaAF and those with PeAF, which appears inconsistent with our previous research and might be accounted by differences in enrollment criteria: in the present study, PeAF patients who received RFCA had shorter AF duration, were younger and had smaller left atrial diameter, i.e., a lower extent of atrial fibrosis than PeAF patients in the previous study.

Relaxin level was related to rhythm outcomes after RFCA

Although RFCA is an effective treatment for AF, it is limited by AF recurrence. In this cohort study, the rate of AF recurrence after RFCA was 21.4%, which is similar to those in other reports [32] and underscores the clinical relevance of identifying patients at high risk for AF recurrence. Previous studies identified several predictors of AF recurrence after RFCA: type of AF, age, sleep apnea, obesity, left atrial size, hypertension, pulmonary vein re-conduction, inflammation, electroanatomical mapping, left atrial pressures and microRNA levels [2, 33–35]. However, measurement of the latter parameters has drawbacks such as invasive manipulation, impreciseness and low predictive value which limit their clinical application. We therefore sought a stable, reliable and readily determined biomarker that could be used to predict effectively recurrence after RFCA, and the present study provides first evidence for serum relaxin level pre-RFCA as such a new risk factor. Although renal insufficiency was one of the important risks for AF recurrence after RFCA [36, 37], we found it was not a significant predictor which may be related to the enrollment criteria of no renal impairment in this study.

We infer that the synthesis and secretion of relaxin may be reflective of increased pro-fibrotic environment such as in AF and heart failure. Furthermore, relaxin can be a cardiac protective factor to relieve heart structural remodeling, stratify and even treat AF in clinical practice. In some animal experiments, relaxin had been demonstrated to reduce AF inducibility, reverse atrial fibrosis and hypertrophy, increase slow, proarrhythmic conduction velocity by increasing sodium current density and decrease action potential duration in spontaneously hypertensive rat and aged rat hearts [26, 30]. In clinic, AF and atrial fibrosis are contacted, influenced and have cause-and-effect relation with each other. However, no effective therapies have been developed to reverse atrial fibrosis, thus we consider that relaxin may provide promising therapy in this unmet clinical need through antifibrosis to achieve antiarrhythmic effect, unlike all other classes of antiarrhythmic therapies. In the RELAX-AHF trial, over 500 patients received serelaxin therapy and 41% of them were in AF at the time of enrollment [28], which showed serelaxin was safe in clinical trials with a high percentage of AF patients. Given previous studies have demonstrated the ability of relaxin to suppress AF and atrial fibrosis, combined with our previous and current studies we infer that in humans, relaxin can reflect the extent of cardiac fibrosis and relaxin is significantly increased in patients with AF and AF recurrence after RFCA. All of these appear to be a rational basis to study relaxin in human AF trials including risk stratification and treatment. Based on the data from clinical trials as well as basic and translational studies, relaxin represents an exciting new and potentially beneficial application prospect in AF.

There are some limitations to this study. Firstly, we did not distinguish between atrial fibrosis and ventricular fibrosis, and the latter may affect relaxin level. Secondly, the incidence of AF recurrence might be underestimated despite the routine screening of patients via outpatient visits and ECG. Thirdly, this analysis was restricted to the measurement of relaxin only once before RFCA and the study did not investigate other biomarkers especially inflammatory markers. Although several studies demonstrated the association of inflammatory markers with AF recurrence after RFCA and serum relaxin levels [38, 39], we did not evaluate it in this study. Fourth, the anteroposterior left atrial dimension provided by M-mode and used for the determination of LAD in our analyses may not reflect true atrial size. Although the left atrial generally enlarges in a spherical fashion, symmetrical enlargement does not always occur; thus, volumetric assessment of left atrial size is a more accurate measure of true atrial size. Finally, this is a single center study and generalizability to other cohorts remains unknown.

In conclusion, this is the first study to reveal the association between pre-RFCA serum relaxin level and post-RFCA AF recurrence. Relaxin level was higher in patients with AF recurrence, and high relaxin level was an independent predictor of post-RFCA AF recurrence. Large-scale, prospective multicenter studies are warranted to further elucidate the association between relaxin level and AF recurrence.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose. No commercial party having a direct or indirect interest in the subject matter of this research conferred a benefit on the authors or on any organization with which the authors are associated. This material has not previously been presented in any form.

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