Interleukin-22 is elevated in the atrium and plasma of patients with atrial fibrillation and increases collagen synthesis in transforming growth factor-β1-treated cardiac fibroblasts via the JNK pathway

YONGXIN WU[1,2*], LIHUA TAN[1,2*], LEI SHI[1], ZICONG YANG[1], YAN XUE[1], TAO ZENG[1], YING SHI[1], YINGZHONG LIN[1] and LING LIU[1]

[1]Department of Cardiology, The People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, Guangxi Zhuang Autonomous Region 530021; [2]Department of Cardiology, Gongan County People's Hospital, Jingzhou, Hubei 434300, P.R. China

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Abstract. Our previous studies demonstrated that interleukin (IL)-22 is involved in cardiovascular diseases such as hypertension, cardiac fibrosis and aortic dissection. The purpose of the present study was to detect IL-22 expression in patients with atrial fibrillation (AF). Atrial tissue was collected from donors with sinus rhythm and patients with permanent AF, and the expression level of IL-22 and its receptors (IL-22R1 and IL-10R2) in both the left atrium (LA) and right atrium (RA) of each sample was detected. Blood samples were also obtained from donors with paroxysmal, persistent and permanent AF and from donors without AF history, and IL-22 levels were measured. In addition, the effects of IL-22 on collagen synthesis in TGF-β1-treated cardiac fibroblasts were investigated. IL-22R1, IL-10R2 and IL-22 expression was elevated in both the LA and RA in permanent AF patients. Elevated IL-22 expression positively correlated with the collagen areas and fibrosis marker levels in the atria of these patients. Plasma IL-22 levels were higher in AF patients compared with healthy donors and increased with increasing AF duration (from paroxysmal to persistent to permanent AF). A positive correlation was observed between IL-22 levels and TGF-β1 levels in AF patients. *In vitro*, recombinant mouse IL-22 treatment upregulated α-SMA, collagen I and collagen III expression in TGF-β1-treated cardiac fibroblasts. These effects were reversed by SP600125, an inhibitor of the JNK pathway. To conclude, IL-22 levels are elevated in patients with AF and may exacerbate collagen synthesis in TGF-β1-induced cardiac fibroblasts. IL-22 may also influence AF by activating the JNK pathway.

Correspondence to: Dr Yingzhong Lin or Dr Ling Liu, Department of Cardiology, The People's Hospital of Guangxi Zhuang Autonomous Region, 6 Taoyuan Road, Nanning, Guangxi Zhuang Autonomous Region 530021, P.R. China
E-mail: yingzhonglin@126.com
E-mail: gxliu@126.com

*Contributed equally

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Introduction

Atrial fibrillation (AF) is a clinical arrhythmia with an incidence of <1% in people <60 years, >12% in people >60 years and >20% in people >85 years (1,2). The occurrence of AF significantly increases the risk of death from underlying heart disease up to 3-fold in patients with chronic heart failure (3,4). AF also results in hemodynamic disorders and thrombosis, and arterial embolism caused by thrombus detachment can result in severe clinical consequences, especially stroke (3,4). Patients with AF benefit from medication and surgery; however, the overall prognosis remains poor, especially in older patient. Therefore, discovery of novel therapeutic targets to prevent the progression of AF is necessary.

It has been revealed that interleukin (IL) family members serve critical roles in the occurrence and progression of AF (5). A previous study reported that polymorphism of the IL-1 receptor antagonist gene allele 2 resulted in a poorly restricted inflammatory response and promoted the presence of lone AF (6). However, improved cardioversion outcomes were reported in AF patients with low serum IL-2 levels compared with those with high serum IL-2 levels (7). The -174G/C IL-6 polymorphism was reported to increase the incidence of AF in patients who underwent coronary artery bypass surgery (8). In addition, anti-IL-17A monoclonal antibodies markedly decrease the development of AF induced by transesophageal burst pacing, while elevated circulating IL-17A levels increased the recurrence rate of AF following catheter ablation (9,10). The G allele and the GG genotype of the IL-27 gene rs153109 polymorphism significantly elevated AF susceptibility in the Chinese Han population (11).

IL-22 is a multifunctional cytokine and its expression is mainly affected by inflammation. It is involved in a variety of biological processes, including inflammatory reactions, oxidative stress, apoptosis, autophagy, cell migration and endothelial dysfunction (12-16). Previous studies revealed that IL-22 regulates the progression of a variety of cardiovascular diseases, including hypertension, cardiac fibrosis, aortic dissection, atherosclerosis and viral myocarditis (16-21). However, the expression of IL-22 in patients with AF is yet to be elucidated. Therefore, in the present study, the expression of IL-22 in atrial tissue and plasma from patients with AF was measured and the effects of IL-22 on transforming growth factor β1 (TGF-β1)-treated cardiac fibroblasts were determined.

Materials and methods

Sample collection. For atrial tissue sample collection, patients with valvular and rheumatic heart disease who had undergone heart valve replacement were enrolled in the present study. Based on their AF histories and the results of a 12-lead electrocardiogram, the patients (n=56) were divided into a permanent AF group (n=35), which included patients who had experienced AF for >6 months, and a sinus rhythm group (n=21), which included patients without a history of AF. No significant pathological changes were observed in the atrial tissue structure in patients of the sinus rhythm group. Both the left and right atrial appendages were collected by cardiac surgeons at the time of surgery and then divided into two subsets. One subset was fixed in 4% paraformaldehyde, and the other subset was rapidly cooled in liquid nitrogen and transferred to a -80˚C freezer for preservation.

For blood sample collection, consecutive patients with AF were prospectively enrolled in the present study. Among all patients, those who had a history or were recently found to suffer from diseases that affect IL-22 expression, including chronic kidney failure, tumors, coronary heart disease, chronic heart failure, connective tissue disease, additional arrhythmias and cardiac structural abnormalities, were excluded from the present study. Patients with other diseases, such as connective tissue disease, that affect IL-22 secretion were also excluded.

Dependent on the duration of AF, the remaining patients (n=155) were divided into paroxysmal AF (n=35), persistent AF (n=45) and permanent AF (n=75) groups, according to the American College of Cardiology/American Heart Association/Heart Rhythm Society AF guidelines published in 2014 (22-24). AF lasting <7 days was defined as paroxysmal AF, AF lasting >7 days that was successfully converted into sinus rhythm was defined as persistent AF and AF lasting >7 days that could not be converted into sinus rhythm was defined as permanent AF. Additional donors without history of AF (n=40) were used as controls. All blood samples were obtained from the ulnar vein by nurses and were then centrifuged (4,000 x g, 4˚C, 15 min) and stored at -80˚C.

All patients provided written informed consent. All samples were collected at the People's Hospital of Guangxi Zhuang Autonomous Region. The study protocol was approved by the Medical Ethics Committee of the People's Hospital of Guangxi Zhuang Autonomous Region (Nanning, China; approval no. 201725GX-H).

Western blot analysis. Human atrial tissue was dissolved in RIPA buffer [50 mM; Tris (pH 7.4), 150 mM NaCl, 1% TritonX-100, 1% sodium deoxycholate, 0.1% SDS, 2 mM sodium pyrophosphate, 25 mM odglycerophosphate, 1 mM EDTA, 1 mM Na_3VO_4, 0.5 μ/ml leupeptin 25˚C] and further lysed at 5,000 Hz using an Ultrasonic Lysimeter (model, XL-2000; Misonix). Subsequently, the atrial tissue homogenate was centrifuged at 6,000 x g at 4˚C for 15 min. The supernatant of each sample was collected and total protein concentration was detected using a bicinchoninic acid Protein Assay kit (Thermo Fisher Scientific, Inc.). A total of 30 μg protein with different molecular weights were separated via electrophoresis on a 10% gel. After transferring the separated proteins to Immobilon-FL PVDF membranes (EMD Millipore) at 200 mA for 1 h, the blots were blocked with 5% nonfat milk (25˚C, 1 h) and incubated with antibodies against IL-22R1 (1:1,000; cat. no. GTX16978; GeneTex, Inc.), IL-10R2 (1:1,000; cat. no. GTX00131-pro; GeneTex, Inc.), IL-22 (1:1,000; cat. no. GTX109659; GeneTex, Inc.) and GAPDH (1:1,000; cat. no. 5174; Cell Signaling Technology, Inc.) at 4˚C for 10 h. Following incubation with goat anti-rabbit IgG H&L Alexa Fluor 647 secondary antibody (1:10,000; cat. no. 150079; Abcam) at 25˚C for 1 h, the blots were scanned using the Odyssey Imaging System (LI-COR Biosciences).

Detection of collagen expression in atria. After fixation at 25˚C for 4 days, the atrial tissue was removed from 4% paraformaldehyde and embedded in paraffin. Subsequently, the atria were cut into 5-6 mm slices, and picrosirius red staining (25˚C; 1 h) was performed to detect the fibrotic areas in the atrium of each sample using a light microscope at x200 magnification. Collagen expression was quantified using ImageJ software v1.8.0 (National Institutes of Health).

Detection of fibrosis marker mRNA expression. Atrial tissue was cut into small pieces and lysed using 1 ml TRIzol® Reagent (cat. no. 15596026; Invitrogen; Thermo Fisher Scientific, Inc.) per 100 mg tissue according to the manufacturer's instructions. Cardiac fibroblasts were also lysed using TRIzol® Reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Total mRNA was collected and the concentration was determined using a NanoDrop™ 2000C spectrophotometer (Thermo Fisher Scientific, Inc.). mRNA was reverse transcribed into cDNA using a Transcriptor First Strand cDNA Synthesis kit (cat. no. 04896866001; Roche Diagnostics) at 92˚C for 5 min. PCR amplification was performed to detect the mRNA levels of TGF-β1, collagen I, collagen III and α-smooth muscle actin (SMA) using the FastStart Universal SYBR Green Mastermix (cat. no. 04913914001; Roche Diagnostics). The following thermocycling conditions were used for the PCR: 35 cycles at 92˚C for 30 sec, 58˚C for 40 sec, and 72˚C for 35 sec. Gene expression levels were normalized to GAPDH levels using the $2^{-\Delta\Delta Cq}$ method (25). Primers were purchased from Tsingke Biological Technology Co., Ltd., and the sequences are listed in Table I.

Measurement of plasma IL-22 and TGF-β1 levels. Blood samples were thawed at 4˚C. Plasma IL-22 and TGF-β1 levels in each sample were then investigated using IL-22

Table I. Sequences of primers used in reverse transcription-quantitative PCR.

Species	Gene	Forward primer (5'→3')	Reverse primer (5'→3')
Human	TGF-β1	TGCTTCAGCTCCACAGAAA	GTATCCAGGCTCCAGATGTAAG
Human	Collagen I	GAGGGCCAAGACGAAGACATC	CAGATCACGTCATCGCACAAC
Human	Collagen III	CTGGACCCCAGGGTCTTC	CATCTGATCCAGGGTTTCCA
Human	GAPDH	GAAGGTGAAGGTCGGAGTC	CTGGGTGGCAGTGATGGCATGG
Mouse	α-SMA	TCCTGACGCTGAAGTATCCGATA	GGCCACACGAAGCTCGTTAT
Mouse	Collagen I	TGGTACATCAGCCCGAAC	GTCAGCTGGATAGCGACA
Mouse	Collagen III	ACGTAGATGAATTGGGATGCAG	GGGTTGGGGCAGTCTAGTC
Mouse	GAPDH	AACTTTGGCATTGTGGAAGG	CACATTGGGGGTAGGAACAC

TGF-β1, transforming growth factor-β1; α-SMA, α-smooth muscle actin.

(cat. no. BMS2047; Invitrogen; Thermo Fisher Scientific, Inc.) and TGF-β1 (cat. no. PHG9211; Invitrogen; Thermo Fisher Scientific, Inc.) ELISA kits, according to the manufacturer's instructions.

Cell culture and treatment. Primary mouse cardiac fibroblasts were purchased from ScienCell Research Laboratories (cat. no. M6300) and cultured in DMEM/F12 (cat. no. A4192101; Gibco; Thermo Fisher Scientific, Inc.) with 10% FBS (cat. no. 16140071; Gibco; Thermo Fisher Scientific, Inc.). Following culture in serum-free DMEM/F12 for 12 h, the cardiac fibroblasts were treated with vehicle (DMSO; cat. no. D12345; Invitrogen; Thermo Fisher Scientific, Inc.), recombinant mouse IL-22 (10 ng/ml; cat. no. AF-210-22-2; PeproTech, Inc.), JNK pathway inhibitor SP600125 (30 μM; cat. no. tlrl-sp60; InvivoGen) or recombinant TGF-β1 (10 ng/ml; PeproTech, cat. no. AF-100-21M-2; PeproTech, Inc.) (17,26,27), and saline treatment as control. Following treatment at 37˚C for 12 h, fibrosis expression in cardiac fibroblasts was detected. First, a subset of cardiac fibroblasts were seeded on glass coverslips. After fixation with 4% paraformaldehyde at 25˚C for 15 min, cells were stained with anti-α-SMA antibody (1:200; cat. no. ab32575; Abcam) at 25˚C and Alexa 568-conjugated secondary antibody (1:200; cat. no. ab175473; Abcam) at 25˚C for 2 h. Stained cells were observed using a fluorescence microscope (Olympus Corporation) at x400 magnification.

Statistical analysis. SPSS 23.0 (IBM Corp.) was used to analyze the data. Continuous variables are expressed as the mean ± SD. Unpaired Student's t-test was used to analyze differences between two groups, while one-way ANOVA followed by the Tukey's post-hoc test was performed to analyze differences between three or more groups. Classification variables are expressed as percentages and were compared using Fisher's exact test. The correlation between IL-22 expression and fibrosis marker expression was analyzed using Spearman's correlation analysis. Linear regression was performed to determine whether IL-22 or TGF-β1 levels were associated with the occurrence of AF. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics. Among the patients who provided atrial samples, longer durations of AF and larger left atrial dimensions were found in the permanent AF group compared with the sinus rhythm group. No differences were observed in other clinical characteristics, including sex, age, incidence of hypertension, type 2 diabetes, basic heart disease, valve disease, left ventricular (LV) end-diastolic diameter and LV ejection fraction. The clinical characteristics of these two groups are listed in Table II.

Among the blood sample donors, those in the permanent AF group were older compared with the control group. The duration of AF gradually increased from the control group to the paroxysmal AF group, to the persistent AF group to the permanent AF group. In addition, the left atrium (LA) dimensions and LV end-diastolic diameter were larger and the LV ejection fraction was lower in the permanent AF group compared with the other three groups. Other characteristics, including sex, incidence of smoking, hypertension, type 2 diabetes and basic heart disease, did not differ between the four groups. The clinical characteristics of each group are listed in Table III.

IL-22 levels are increased in the atria of patients with permanent AF. Western blotting results indicated that IL-10R2, IL-22R1 and IL-22 levels significantly increased in both the LA and right atrium (RA) in the permanent AF group compared with the sinus rhythm group (Fig. 1A). Increased trends of IL-22 levels were observed in the collagen area (Fig. 1B). Atrial IL-22 expression positively correlated with the atrial collagen area percentage in patients with permanent AF (Fig. 1C). In addition, levels of fibrosis-related signaling pathway members and fibrosis markers, such as TGF-β1, collagen I and collagen III, significantly increased in both the LA and RA of patients with permanent AF compared with the sinus rhythm group (Fig. 1D-F). Positive correlations were observed between elevated TGF-β1, collagen I and collagen III mRNA levels and increased IL-22 expression (Fig. 1G-I).

Plasma IL-22 levels are elevated in patients with AF. As demonstrated by ELISA, plasma IL-22 levels were significantly higher

Table II. Clinical characteristics of patients in the sinus rhythm and permanent AF groups.

Characteristic	Sinus rhythm (n=21)	Permanent AF (n=35)	P-value
Male, n (%)	12.0 (57.1)	25.0 (71.4)	0.383
Age (years)	55.2±11.2	57.6±14.0	0.411
Smoking, n (%)	7.0 (33.3)	19.0 (54.3)	0.170
Hypertension, n (%)	6.0 (28.6)	19.0 (57.1)	0.096
Type 2 diabetes, n (%)	3.0 (14.3)	12.0 (34.3)	0.128
Duration of AF (days)	0.0	149.2±88.5	<0.001
Basic heart disease			
Rheumatic heart disease (n, %)	14.0 (66.7)	28.0 (80.0)	0.343
Valvular heart disease, n (%)	7.0 (33.3)	7.0 (20.0)	0.343
Diseased valve			
Mitral valves involved, n (%)	9.0 (42.9)	17.0 (48.6)	0.785
Aortic valves involved, n (%)	5.0 (23.8)	9.0 (25.7)	0.999
Combined valvular disease, n (%)	7.0 (33.3)	9.0 (25.7)	0.557
Cardiac structure			
LA dimension (mm)	36.5±4.6	49.1±11.1	<0.001
LV end-diastolic diameter (mm)	44.1±4.7	46.9±6.7	0.099
LV ejection fraction (%)	56.9±4.7	55.1±4.2	0.143

Smoking refers to current smoking status and less than 6 months of abstinence. Data are also presented as the mean ± standard deviation. AF, atrial fibrillation; LA, left atrium; LV, left ventricle.

Table III. Clinical characteristics of non-AF donors and patients with AF.

		AF		
Characteristic	Non-AF	Paroxysmal	Persistent	Permanent
Male, n (%)	22.0 (55.0)	23.0 (65.7)	30.0 (66.7)	53.0 (70.7)
Age (years)	58.1±12.8	61.4±14.0	64.3±15.4	66.3±15.2[a]
Smoking, n (%)	11.0 (27.5)	15.0 (42.9)	19.0 (47.5)	32.0 (42.6)
Hypertension, n (%)	13.0 (32.5)	14.0 (40.0)	21.0 (52.5)	31.0 (42.7)
Type 2 diabetes (n,%)	3.0 (7.5)	2.0 (5.7)	4.0 (10.0)	7.0 (9.3)
Duration of AF (days)	0.0	2.0±0.7[a]	4.7±1.9[a,b]	128.1±56.2[a-c]
Basic heart disease				
Rheumatic heart disease, n (%)	13.0 (32.5)	9.0 (25.7)	11.0 (27.5)	33.0 (44.0)
Valvular heart disease, n (%)	6.0 (15.0)	4.0 (11.4)	9.0 (22.5)	13.0 (17.3)
Dilated cardiomyopathy, n (%)	7.0 (17.5)	7.0 (20.0)	5.0 (12.5)	11.0 (14.7)
Hypertrophic cardiomyopathy, n (%)	6.0 (15.0)	3.0 (8.6)	8.0 (20.0)	8.0 (20.0)
Others, n (%)	8.0 (20.0)	8.0 (20.0)	7.0 (17.5)	11.0 (14.7)
Cardiac structure				
LA dimension (mm)	33.7±5.1	32.8±6.2	34.1±6.4	39.1±6.6[a-c]
LV dimension (mm)	47.6±7.2	49.3±6.4	49.3±7.2	52.1±7.1[a-c]
LV ejection fraction (%)	59.7±5.3	58.8±4.7	59.1±4.5	54.6±5.9

Data are presented as the mean ± standard deviation. [a]P<0.05 vs. control group; [b]P<0.05 vs. paroxysmal AF group; [c]P<0.05 vs. persistent AF group. AF, atrial fibrillation; LA, left atrium; LV, left ventricle.

in all AF groups compared with the non-AF group (Fig. 2A). IL-22 levels gradually increased with increasing AF duration (from the paroxysmal AF group to the persistent AF group to the permanent AF group) (Fig. 2A). Circulating TGF-β1 levels

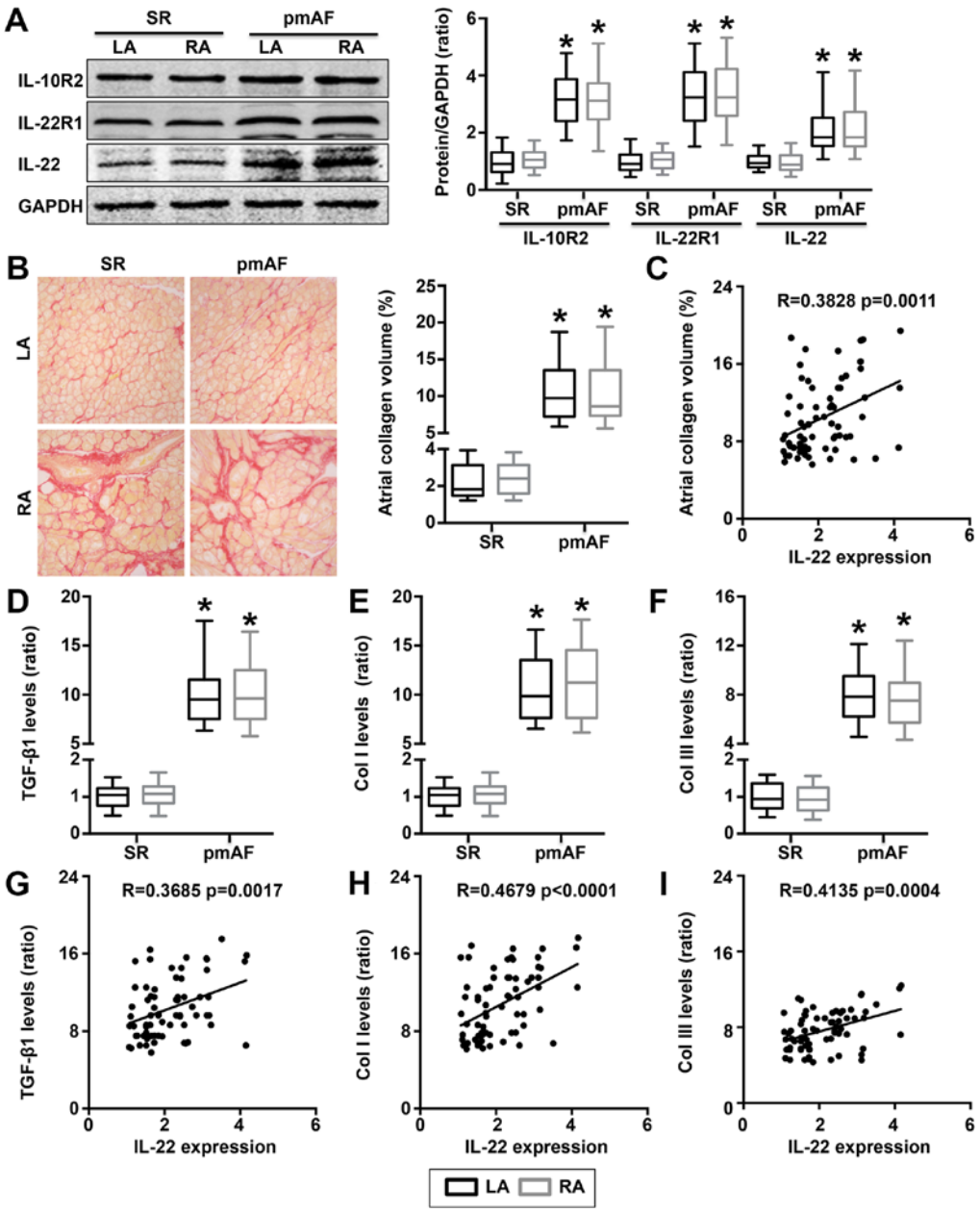

Figure 1. Atrial IL-22 expression in patients with pmAF. (A) IL-10R2, IL-22R1 and IL-22 expression in both the LA and RA of the SR and pmAF patient groups. (B) Atrial collagen volume in the LA and RA in the two groups were quantified by Image J softwore after magnification by 400 by a light microscope. (C) Correlation between atrial IL-22 and atrial collagen area. (D) TGF-β1, (E) collagen I and (F) collagen III mRNA expression in the LA and RA of each group of all AF patients. Correlation between atrial (G) TGF-β1, (H) collagen I and (I) collagen III mRNA expression and atrial IL-22 expression. *P<0.05 vs. the SR group. AF, atrial fibrillation; SR, sinus rhythm; pmAF, permanent atrial fibrillation; IL, interleukin; LA, left atrium; RA, right atrium; Col I, collagen I; Col III, collagen III; TGF-β1, transforming growth factor-β1.

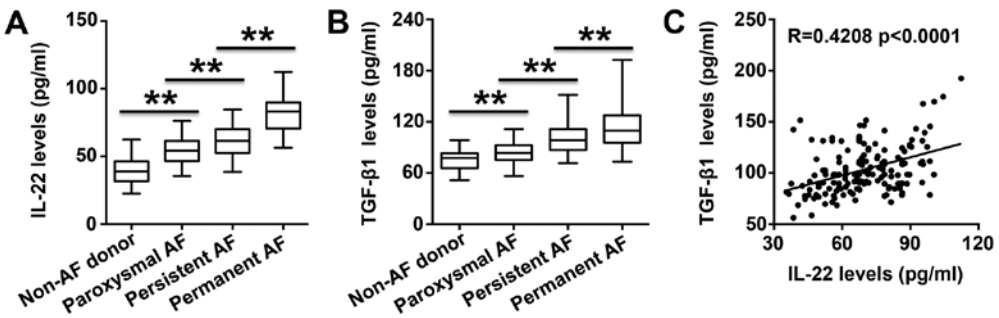

Figure 2. Circulating IL-22 levels in patients with AF. (A) Plasma IL-22 levels in non-AF donor, paroxysmal AF, persistent AF and permanent AF groups. (B) Plasma TGF-β1 levels in each AF group. (C) Correlation between plasma IL-22 levels and plasma TGF-β1 levels in patients with AF. **P<0.01. AF, atrial fibrillation; IL, interleukin; TGF-β1, transforming growth factor-β1.

Table IV. Univariate analysis and multivariate linear regression analysis results.

	Univariate analysis			Multivariate analysis		
Variable	β-value	95% CI	P-value	β-value	95% CI	P-value
IL-22	0.578	0.319-0.837	<0.001	0.276	0.319-0.837	0.008
TGF-β1	0.411	0.257-0.565	<0.001	0.197	0.148-0.246	0.017
Male	0.233	0.174-0.292	0.247			
Age	0.171	0.125-0.217	0.566			
Smoking	0.204	0.152-0.256	0.046	0.094	0.068-0.120	0.392
Hypertension	0.116	0.098-0.134	0.015	0.067	0.037-0.097	0.037
Type 2 diabetes	0.098	0.079-0.117	0.174			
LA dimension	0.218	0.161-0.275	0.025	0.103	0.071-0.135	0.059

Smoking refers to current smoking status and less than 6 months of abstinence. CI, confidence interval; IL, interleukin; TGF-β1, transforming growth factor-β1; LA, left atrium.

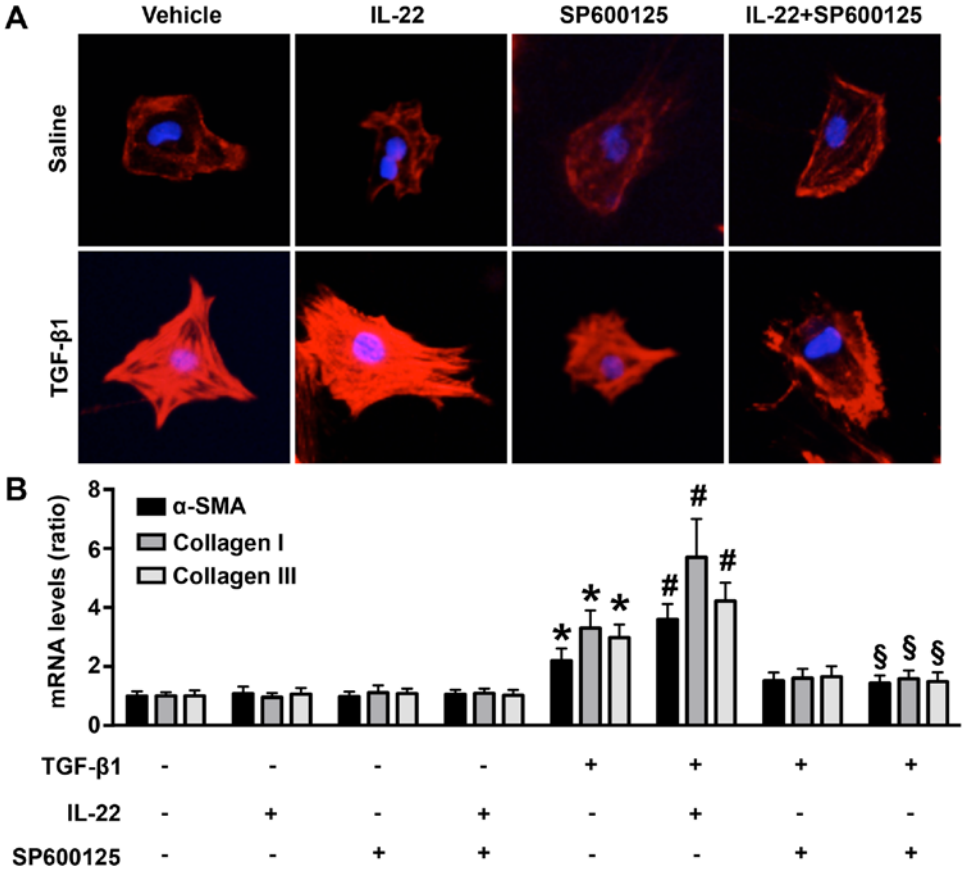

Figure 3. Effect of IL-22 on collagen synthesis. (A) Cardiac fibroblasts treated with vehicle, TGF-β1, IL-22 and/or SP600125. (B) Collagen I, collagen III and α-SMA mRNA expression in each group. n=5. *P<0.05 vs. vehicle group; #P<0.05 vs. TGF-β1-treated group; §P<0.05 vs. TGF-β1 + IL-22-treated group. IL, interleukin; TGF-β1, transforming growth factor-β1; α-SMA, α-smooth muscle actin.

also revealed similar trends to plasma IL-22 levels (Fig. 2B). A positive correlation between TGF-β1 levels and IL-22 levels was demonstrated in all patients with AF (Fig. 2C).

IL-22 expression is associated with AF. Clinical characteristics which were demonstrated with fibrosis or related to the presence of AF, including IL-22 levels, TGF-β1 levels, sex, age, incidence of smoking, hypertension, and type 2 diabetes and the left atrial dimension, were included in univariate analysis. IL-22 levels, TGF-β1 levels, smoking incidence, hypertension incidence and left atrial dimension may be related to the presence of AF. These variables were used for further multivariate linear regression analysis. The results demonstrated that both plasma IL-22 and TGF-β1 levels were independently

associated with the presence of AF. The β, 95% confidence interval and P-values for each variable are listed in Table IV.

Recombinant IL-22 treatment upregulates collagen synthesis in TGF-β1-treated cardiac fibroblasts. In vitro, TGF-β1 treatment markedly increased the expression of α-SMA in cardiac fibroblasts, indicating the buildup of fibrosis following TGF-β1 treatment. These effects were increased by IL-22 treatment, but partially reversed by SP600125 treatment (Fig. 3A). In addition, TGF-β1 treatment significantly increased the mRNA expression levels of the fibrosis markers α-SMA, collagen I and collagen III in fibroblasts compared with groups not treated with TGF-β1 (Fig. 3B). The addition of exogenous IL-22 further increased fibrosis marker mRNA expression compared with TGF-β1 alone (Fig. 3B). The pro-fibrotic effect of IL-22 was partially reversed by SP600125 treatment (Fig. 3B). Neither IL-22 nor SP600125 affected collagen synthesis in cardiac fibroblasts without TGF-β1 treatment (Fig. 3B).

Discussion

The present study examined the expression of IL-22 in patients with AF and investigated the association between IL-22 and fibrosis. Atrial IL-22 levels were elevated and positively correlated with atrial fibrosis in permanent AF patients. In addition, circulating IL-22 levels were also elevated and positively correlated with TGF-β1 levels in patients with AF. Moreover, *in vitro*, IL-22 treatment increased TGF-β1-induced collagen fiber expression in mouse cardiac fibroblasts, and this effect was partially restored by a JNK pathway inhibitor.

IL-22 is an immune-related cytokine secreted primarily by macrophages and $CD4^+$ T lymphocytes. An increasing number of studies have reported that IL-22 expression is increased in inflammation-mediated cardiovascular disease (16-21). Previous studies reported that both T helper (Th) 22 and IL-22 levels were increased in Coxsackie virus B3-induced acute and chronic viral myocarditis and dilated cardiomyopathy (26,27). In our previous study, circulating Th22/IL-22 levels were elevated in patients with acute coronary syndrome (28). In addition, animal studies revealed that circulating Th22 and IL-22 levels and cardiac IL-22 and IL-22R1 levels were higher than baseline levels in angiotensin II-infused mice (15,16). In clinical experiments, both circulating IL-22 levels and aortic IL-22 expression were increased in patients with human aortic dissection (17,18). The present study obtained atrial tissue from patients with AF and detected the expression of IL-22 and IL-22 receptors, including IL-10R2 and IL-22R1. The current results revealed that IL-10R2, IL-22R1 and IL-22 were all upregulated in the atrial tissue of patients with AF. Similarly, it was demonstrated that circulating IL-22 levels were elevated in patients with AF. The current findings of elevated circulating IL-22 and atrial IL-22 levels suggest that increased IL-22 expression is associated with AF.

Although the specific mechanisms underlying AF are yet to be elucidated, multiple pathological factors were revealed to influence the occurrence of AF, including inflammatory responses, oxidative stress and left atrial apoptosis (8,9,29,30). These injury factors may result in atrial remodeling, including atrial structural changes and atrial fibrosis, which can cause electrical remodeling and ultimately contribute to the onset and progression of AF (31). Although it has been reported that delaying atrial remodeling reduces the occurrence and duration of AF (31,32), a recently published long-term clinical follow-up study revealed that both atrial fibrosis and atrial structural remodeling affected the success rate and postoperative recurrence rate of AF following radiofrequency ablation (32). These indicates that electrical remodeling induced by atrial fibrosis may represent the primary cause of the occurrence and development of AF (32). In fact, increasing studies have focused on atrial fibrosis as a mechanism for atrial fibrillation, as data from animal studies and clinical experiments have revealed that severe atrial fibrosis can result in a higher incidence and duration of AF; in addition, the hypothesis that inhibition of atrial fibrosis significantly inhibits the occurrence and progression of AF has been increasingly accepted (33-35).

Inflammatory response-mediated fibrosis has been widely researched, and the participation of inflammatory cytokines in the progression of AF via mediation of atrial fibrosis has been well documented (8,9). Furthermore, increased atrial inflammatory responses have been associated with increased risk of recurrent AF during follow-up (36). Although the regulatory effect of IL-22 on the fibrotic process was confirmed in previous studies (16-19), the association between IL-22 and fibrosis in patients with AF was further investigated in the present study. It was evidenced that IL-22 expression positively correlates with TGF-β1, collagen I and collagen III expression in the atrium, and with TGF-β1 levels in plasma. According to the current results, IL-22 may participate in AF progression via regulating atrial fibrosis. In addition, the results of linear regression analysis indicated that both IL-22 and TGF-β1 were associated with the occurrence of AF, further supporting the aforementioned hypothesis.

IL-22 initiates downstream signaling pathways via binding to IL-22 receptors, including IL-10R2 and IL-22R1, on target cells, thereby regulating multiple biological effects, including inflammation, oxidative stress, apoptosis and autophagy (15). A variety of immune and non-immune cells are targets of IL-22, including cardiac fibroblasts (14). The downstream signaling pathways of IL-22 include the STAT3 and mitogen-activated protein kinase (ERK, JNK and P38) pathways. Previous research has demonstrated that IL-22 regulates fibrosis via the JNK pathway (16,37). Therefore, to further explore the possible mechanisms underlying IL-22 regulation of AF, the effects of exogenous IL-22 on collagen synthesis in TGF-β1-treated cardiac fibroblasts were investigated. In addition, SP600125 was used to block the JNK signaling pathway in cardiac fibroblasts. It was revealed indicate that IL-22 may further increase TGF-β1-induced collagen synthesis and that this effect was inhibited by treatment with SP600125. These findings indicate that IL-22 activates the JNK signaling pathway to increase fibrosis *in vitro*, and also indicate that IL-22 increases atrial fibrosis via activation of the JNK pathway during inflammatory response, thereby regulating the progression of AF. However, animal studies still need to be performed to confirm this hypothesis.

In summary, the present study revealed that IL-22 expression significantly increased in both the plasma and atrial tissue of patients with AF compared with non-AF individuals, and that elevated IL-22 expression positively correlated with the expression of fibrosis-related markers. The results of the *in vitro* studies suggested that IL-22 may regulate fibrosis via the JNK

signaling pathway. The present results also indicate that IL-22 is closely associated with the occurrence of AF and may represent a potential target for the clinical treatment of this condition. However, the present research had certain limitations. Firstly, the sample size of specimens, particularly atrial tissue samples, was small; more patients should be included in future studies. In addition, while the present results indicated that IL-22 may participate in AF via regulating atrial fibrosis; the specific mechanisms have not been investigated, and further *in vivo* research is necessary to confirm this hypothesis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LL and YL conceived and designed the study; TZ and YS collected the samples; YW, LT, LS, YX, YS and ZY performed the experiments; TZ and YX analyzed the data; TZ and YS were involved in drafting the manuscript or revising it critically for important intellectual content; YW and YL reviewed and edited the manuscript; YS and ZY gave final approval of the version to be published. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Medical Ethics Committee of the People's Hospital of Guangxi Zhuang Autonomous Region (Nanning, China; approval no. 201725GX-H).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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