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ORIGINAL RESEARCH

Heart Failure, Female Sex, and Atrial Fibrillation Are the Main Drivers of Human Atrial Cardiomyopathy: Results From the CATCH ME Consortium

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BACKGROUND: Atrial cardiomyopathy (atCM) is an emerging prognostic factor in cardiovascular disease. Fibrotic remodeling, cardiomyocyte hypertrophy, and capillary density are hallmarks of atCM. The contribution of etiological factors and atrial fibrillation (AF) to the development of differential atCM phenotypes has not been quantified. This study aimed to evaluate the association between histological features of atCM and the clinical phenotype.

METHODS AND RESULTS: We examined left atrial (LA, n=95) and right atrial (RA, n=76) appendages from a European cohort of patients undergoing cardiac surgery. Quantification of histological atCM features was performed following wheat germ agglutinin/CD31/vimentin staining. The contributions of AF, heart failure, sex, and age to histological characteristics were determined with multiple linear regression models. Persistent AF was associated with increased endomysial fibrosis (LA: +1.13±0.47 μm, P=0.038; RA: +0.94±0.38 μm, P=0.041), whereas total extracellular matrix content was not. Men had larger cardiomyocytes (LA: +1.92±0.72 μm, P<0.001), while women had more endomysial fibrosis (LA: +0.99±0.56 μm, P=0.003). Patients with heart failure showed more endomysial fibrosis (LA: +1.85±0.48 μm, P<0.001) and extracellular matrix content (LA: +3.07±1.29%, P=0.016), and a higher capillary density (LA: +0.13±0.06, P=0.007) and size (LA: +0.46±0.22 μm, P=0.044). Fuzzy k-means clustering of histological features identified 2 subtypes of atCM: 1 characterized by enhanced endomysial fibrosis (LA: +3.17 μm, P<0.001; RA: +2.86 μm, P<0.001), extracellular matrix content (LA: +3.53%, P<0.001; RA: +6.40%, P<0.001) and fibroblast density (LA: +4.38%, P<0.001), and 1 characterized by cardiomyocyte hypertrophy (LA: +1.16 μm, P=0.008; RA: +2.58 μm, P<0.001). Patients with fibrotic atCM were more frequently female (LA: odds ratio [OR], 1.33, P=0.002; RA: OR, 1.54, P=0.004), with persistent AF (LA: OR, 1.22, P=0.036) or heart failure (LA: OR, 1.62, P<0.001). Hypertrophic features were more common in men (LA: OR=1.33, P=0.002; RA: OR, 1.54, P=0.004).

CONCLUSIONS: Fibrotic atCM is associated with female sex, persistent AF, and heart failure, while hypertrophic features are more common in men.

Key Words: atrial cardiomyopathy ■ atrial fibrillation ■ endomysial fibrosis ■ heart failure

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CLINICAL PERSPECTIVE

What Is New?

- Heart failure, female sex, and history of atrial fibrillation are associated with endomysial fibrosis, while total extracellular matrix content is associated only with heart failure and female sex but not with atrial fibrillation history.
- Cluster analysis of clinical traits and histological characteristics identified 2 distinct types of atrial cardiomyopathy: fibrotic atrial cardiomyopathy associated with female sex, heart failure, and history of atrial fibrillation; and hypertrophic atrial cardiomyopathy associated with male sex.

What Are the Clinical Implications?

- Our findings provide a better understanding of the association between clinical phenotypes and structural characteristics of the atria.

Nonstandard Abbreviations and Acronyms

atCM	atrial cardiomyopathy
CATCH ME	Characterizing Atrial fibrillation by Translating Its Causes Into Health Modifiers in the Elderly
ECM	extracellular matrix content
HTX	heart transplant xenograft
LGE-MRI	late gadolinium-enhancement cardiac magnetic resonance imaging
P_{adj}	adjusted *P* value
PC	principal component
WGA	wheat germ agglutinin

Many cardiovascular diseases substantially impact atrial function. Heart failure, hypertension, supraventricular tachycardias, valvular diseases, and thyrotoxicosis can, over time, deteriorate the mechanical, electrical, and endocrine function of the atria. In some patients, these mechanisms cause a progressive atrial cardiomyopathy (atCM), which, in the consensus paper by Goette et al[1] in 2016, was defined as "any complex of structural, architectural, contractile or electrophysiological changes affecting the atria with the potential to produce clinically relevant manifestations." Importantly, atCM has a substantial impact on cardiac performance, and also on the occurrence of atrial fibrillation (AF) and stroke.[2–4] Once AF occurs, the arrhythmia itself accelerates the progression of atCM.[1,5] atCM mechanisms vary between individual patients and over time,[6] likely contributing to the limited efficacy of rhythm control therapies for AF.[7,8]

One of the key features of atCM is enhanced fibrosis, as extensively reported in multiple animal models[9–17] and various clinical settings, including left/right ventricular dysfunction,[18] AF,[19,20] and heart failure.[21,22] We recently reported that endomysial fibrosis, defined as extracellular matrix deposition between myocytes within the muscle bundles, rather than overall connective tissue content, is the main determinant of conduction disturbances in human AF.[23] Fibroblasts are not only responsible for extracellular matrix (ECM) formation, but they may also interact electrically with cardiomyocytes, thereby affecting conduction.[24,25] Increased cardiomyocyte size relates to increased wall thickness and chamber dilatation, both of which can alter electrical propagation.[26–28] Finally, capillary rarefaction may be a marker of prolonged myocardial stress, as observed in patients with heart failure with preserved ejection fraction, hypertensive heart disease, and AF.[29,30]

Although a first classification of atCM was proposed in the consensus paper from 2016,[1], a detailed investigation of the relationship between histological features of atCM and clinical traits is still missing. Here, we sought to comprehensively appraise histological changes in both right atrial (RA) and left atrial (LA) samples in a large European cohort of patients undergoing cardiac surgery for a variety of indications. We aimed to systematically quantify the type of atrial fibrosis, fibroblast density, myocyte size, capillary density, and capillary size using a previously validated, semiautomated quantification method developed for high-throughput histological studies.[31] Additionally, we aimed to cluster LA and RA samples on the basis of their histological features and to identify the clinical traits associated with these clusters.

METHODS

Patient Selection and Tissue Sampling

Human LA and RA appendage samples were collected from the Characterizing Atrial fibrillation by Translating Its Causes Into Health Modifiers in the Elderly (CATCH ME; www.catch-me.info) atrial tissue biobank of 227 patients undergoing coronary artery bypass grafting, valvular surgery, or heart transplant xenograft (HTX). An RNA sequencing data set of this study population was recently published.[32] A total of 170 tissue samples (n=95 LA, n=76 RA, 20 of which were paired) were of sufficient quality and included in the analyses. The study was performed in accordance with relevant guidelines and was approved by the local

ethical committees of the sampling centers. Included patients provided written informed consent. Due to the sensitive nature of the data collected for this study, and the restrictions regarding data sharing imposed by the data transfer agreement clause of the medical ethical approval forms for this study, no raw data or heart biopsies can be shared with third parties.

Triple Staining and Microscopy

Snap-frozen tissue samples were cryosectioned (6 μm) and stained with wheat germ agglutinin (WGA), anti-CD31, and anti-vimentin, as previously described.[31] Acetone-based antigen fixation (10 min) was followed by blocking of nonspecific binding sites with blocking solution (2 m/v% fraction V BSA, 0.3 mol/L glycine) for 60 minutes. Rabbit monoclonal anti-vimentin (1/150, Abcam [ab92547], Cambridge) and mouse monoclonal anti-CD31 (1/50, Abcam [ab9498], Cambridge) were added overnight at room temperature to visualize fibroblasts and endothelial cells. After 3-fold washing with 1x PBS, a mixture of Alexa 594-conjugated WGA (1/200, Thermo Fisher [W11262], Netherlands), goat anti-mouse 488 (1/200, Thermo Fisher [A-10680], Netherlands), and goat anti-rabbit 405 (1/200, ThermoFisher [35550], Netherlands) was applied for 120 minutes. Prolong Gold Antifade mounting medium (Thermo Fisher [P10144], Netherlands) was added when coverslips were mounted. Sections were imaged using a Leica microscope (DM4B) and camera (MC170HD). Nonoverlapping images were obtained manually across the tissue section, without slide-scanning technology, at ×200 magnification. Only sections with transversely cut cardiomyocytes were selected and photographed for further analysis. Size measurements (capillary and cardiomyocyte size) and measurements of endomysial fibrosis (intercellular distance) are more robust in areas with transversely cut cells compared with obliquely cut cells.

Automated Visualization and Quantification of Histological atCM Features

Standardized, automated analysis of structural tissue characteristics was performed using the recently developed and validated custom-built ImageJ plugin JavaCyte.[31] An average of 3 images were investigated from 3 individual tissue slices per patient, totaling 547 WGA/CD31/vimentin–positive images. Cardiomyocytes were detected by segmentation of objects on the basis of the detection of local fluorescence minima. WGA images were thresholded applying Phansalkar's algorithm for adaptive local thresholding (Figure 1). To quantify the overall connective tissue content, the fraction of ECM (total ECM) was calculated as the percentage of WGA-positive pixels. The distance between neighboring cardiomyocytes was quantified as a measure for endomysial fibrosis, as described previously.[31] CD31- and vimentin-positive images were thresholded applying Landini's hue, saturation, and brightmess–based color threshold. Capillary density (capillaries per cardiomyocyte) and capillary minimal Feret diameter were obtained from thresholded CD31 images. The percentage of vimentin signal was obtained from thresholded vimentin-positive images, after correction for CD31–vimentin double-positive pixels to rule out cross reactivity with endothelial cells.

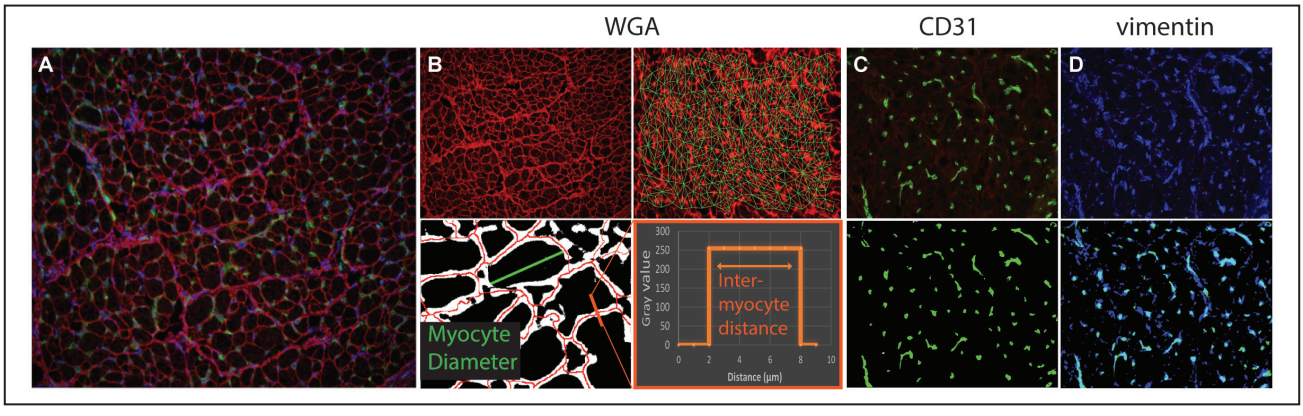

Figure 1. Methodology of immunohistochemical staining and the JavaCyte algorithm for analysis of histological parameters. **A**, Samples are triple-stained with wheat germ agglutinin (WGA), CD31, and vimentin. **B**, After thresholding of the WGA image, the fraction of WGA-positive pixels is a measure for total extracellular matrix (ECM) content. Cell-to-cell distances were calculated as a measure of endomysial fibrosis. A control image is generated visualizing the measured distances between cells. Cardiomyocyte size is measured as the shortest diameter of the cells. Finally, a heat map is generated with a color scale indicating the density of endomysial fibrosis surrounding each cardiomyocyte. Cluster analysis is performed to detect clusters of endomysial fibrosis. **C**, CD31 images are thresholded to count capillaries and normalize for cardiomyocyte count in the corresponding WGA image. Minimal Feret diameter of each capillary is measured. **D**, Vimentin images are thresholded to calculate the fibroblast-to-cardiomyocyte ratio.

Statistical Analysis: Association Between Clinical Traits and Histological Features

The open-access statistical software R version R4.0.1 R Foundation for Stastistical Computing, Vienna, Austria) was used for all analyses.[33] Clinical differences between patients donating an LA biopsy, RA biopsy, or paired biopsy were tested with 1-way ANOVA (continuous dependent variable) or chi-square test (dichotomous dependent variable). To study differences in atrial histology between left and right atria, all samples (n=171) were pooled for analysis using regression models including the covariate tissue location. For this test specifically, paired LA/RA samples (n=20) were excluded. Additionally, the interaction between heart failure and tissue location (LA/RA) was assessed. Next, linear mixed-effects regression models were constructed to study the association between histological features and clinical features, stratified by side, using the R package lme4. Age, sex, rhythm history, and heart failure were explicitly modeled on the basis of a priori knowledge from available literature and unadjusted associations with histological traits. Additional clinical covariates (weight, height, indication for coronary artery bypass grafting surgery, prior myocardial infarction, indication for aortic and mitral valve surgery, tricuspid valve insufficiency, thyroid dysfunction, hypertension, chronic kidney failure, diabetes, and history of stroke or transient ischemic attack) were also included in the model. To prevent overfitting of these models, dimensionality was reduced by transformation of 13 additional clinical traits into four principal components (PCs). The prcomp package[34] was used to scale variables and calculate orthogonal PCs. The proportion of variance accounted for by the first four PCs in the LA and RA samples was 55.8% and 56.6%, respectively, as visualized in a scree plot (Figure S1). Finally, unsupervised fuzzy k-means clustering, as implemented in the R package fclust,[35] was applied on the basis of histological features. The optimum number of clusters was confirmed by inspection of the within sum of squares plot and Silhouette curve (Figure S2). Associations between clinical characteristics and the identified clusters was studied using logistic regression, adjusted for rhythm history, heart failure, sex, and age. Tests of linearity (residuals versus fitted plot), multivariate normality (Q-Q plot and histogram), homoscedasticity (scatter plot) and absence of multicollinearity (correlation matrix) were performed to assess regression validity. Continuous group characteristics are reported as mean±SD, whereas dichotomous traits were noted as number (percentage). Results from regression analyses were noted as the unstandardized β±SE, unless otherwise specified. Finally, odds ratios were noted as OR, 95% CI. To reduce type I error in analyses with multiple outcomes, an additional permutation *P* value was calculated (adjusted *P* value [P_{adj}]) using the R package lmPerm. The number of iterations for calculation of permuted *P* values was set at 100 000.

RESULTS

Tissue Sample Selection

A total of 260 samples from 227 patients were collected from the CATCH ME biobank. Dropout occurred due to low tissue quantity (inability to perform cryosectioning, n=66), poor tissue quality detected by microscopy (n=19), or failure to pass JavaCyte's built-in quality control (n=4; Figure 2). A total of 171 samples (n=95 LA, n=76 RA) were of sufficient quality for analysis. These included 20 paired samples (20 LA+RA) donated by patients undergoing HTX (n=10), mitral valve surgery (n=7), coronary artery bypass grafting surgery (n=1), aortic valve surgery (n=1), or donation without cardiovascular indication (n=1). Clinical and demographic information of the included patients is summarized in Table 1.

Right–Left Differences in Atrial Histology

Explorative unadjusted analysis showed that structural properties of LA and RA samples differed substantially. The right atrium had more endomysial fibrosis (+1.28 μm; $P<0.001$) and a higher total ECM content (+3.77%, $P<0.001$) than LA, but smaller cardiomyocytes (–0.69 μm, $P=0.021$). Nonsignificant trends toward less fibroblast-specific vimentin expression (–8.6%, $P=0.092$) and lower capillary density (–0.05 capillaries/myocyte, $P=0.061$) were observed in the right atrium. Capillary size did not differ between left and right atria ($P=0.284$). Regression analyses adjusted for age, sex, rhythm history, heart failure and atrial biopsy location (after exclusion of paired samples) confirmed the RA/LA differences (Figure 3), including more endomysial fibrosis (+1.51±0.25 μm, $P_{adj}<0.001$), a larger total ECM content (+6.15±1.14%, $P_{adj}<0.001$), lower capillary density (–0.06±0.03 capillaries/myocyte, $P_{adj}=0.039$), larger capillary size (+0.26±0.12 μm, $P_{adj}=0.032$), but smaller cardiomyocytes (–0.92±0.31 μm, $P_{adj}=0.005$) in the right atrium. Moreover, we found a significant negative interaction (–2.52±0.73, $P_{adj}<0.001$) between atrial side and heart failure status on endomysial fibrosis, indicating that heart failure is more strongly associated with endomysial fibrosis in LA than RA samples. Regression assumptions were tested, and no violations were detected. Given the substantial difference in atrial histology between atria, further analyses were stratified by atrial side.

Association Between Clinical Traits and Histological Features of atCM

Explorative simple linear regression analysis, stratified by atrial side, identified candidate traits associated

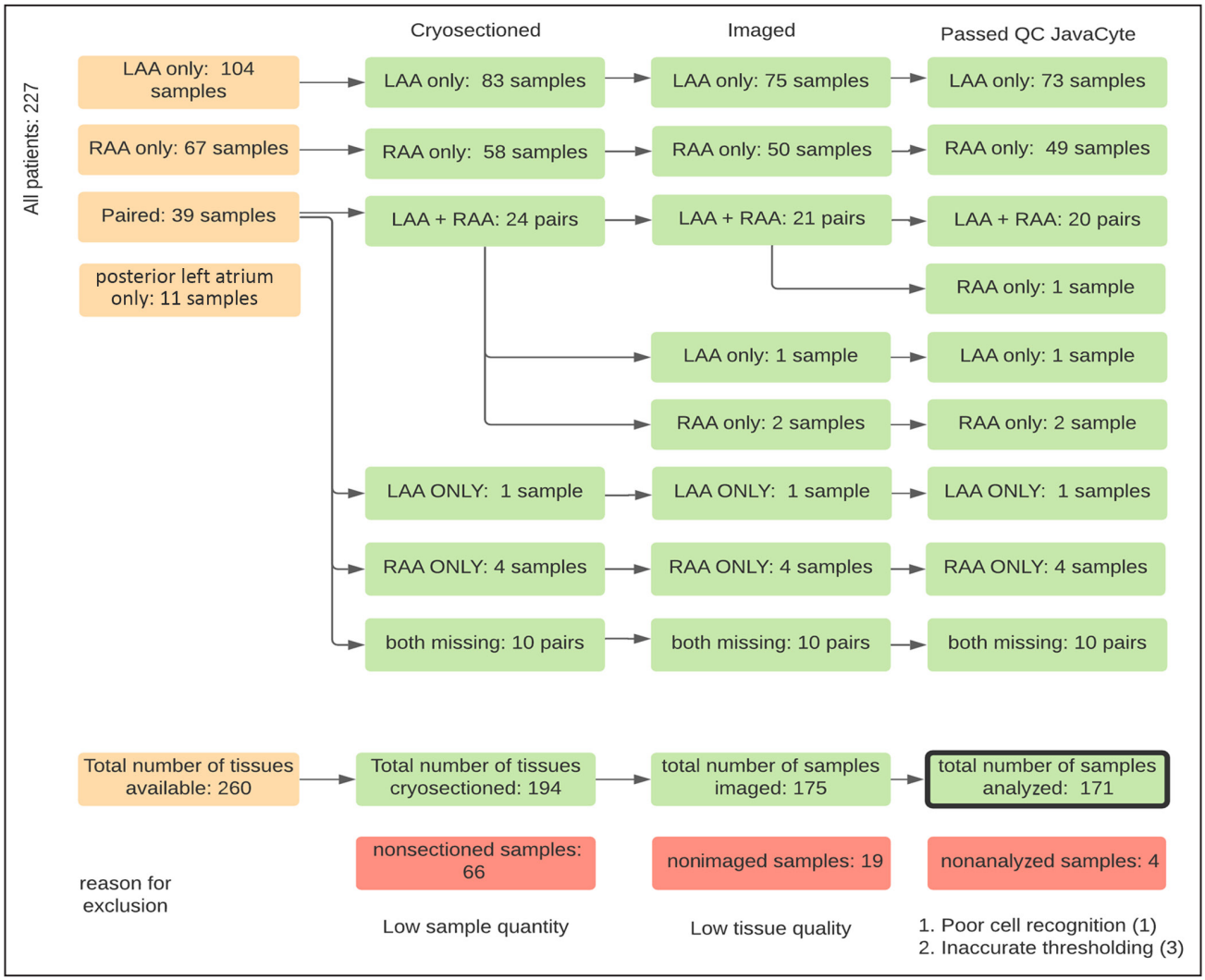

Figure 2. Flowchart of sample selection.
260 left atrial appendage (LAA) or right atrial appendage (RAA) biopsies were collected from 227 patients. Sixty-six samples were not cryosectioned because of low tissue quantity. Nineteen samples were excluded due to poor tissue quality observed during microscopy. An additional 4 samples were excluded as a result of failure to pass built-in quality control (QC) during analysis with JavaCyte. Reasons for exclusion were poor myocyte detection or poor thresholding of images.

with structural remodeling features (Tables S1–S6). Multiple linear regression was performed to study associations between histological features and clinical profiles (Figure 4). Rhythm history, heart failure, sex, and age were explicitly included in the model. No violations of regression validity were detected.

Overall, heart failure, rhythm history, and female sex showed the strongest associations with atrial histology, with stronger associations in the LA than RA (Figure 4). Female sex (β=4.88±1.51, P_{adj}<0.001) and heart failure (β=3.07±1.29, P_{adj}=0.016) were associated with higher ECM content in the left atrium. More endomysial fibrosis was observed in both atria of patients with persistent AF (β_{LA}=1.13±0.47, P_{adj}=0.038; β_{RA}=0.94±0.38, P_{adj}=0.041), in the left atrium of women (β=0.99±0.56, P_{adj}=0.003), and in the left atrium of patients with heart failure (β=1.85±0.48, P_{adj}<0.001). More fibroblast-specific signal was detected in the right atrium of patients with persistent AF (β=4.13±1.58, P_{adj}=0.007) and the left atrium of patients with heart failure (β=3.20±1.14, P_{adj}=0.010). Moreover, male patients had larger cardiomyocytes in the left atrium (β=1.92±0.72, P_{adj}<0.001). Increased capillary density was observed in the left atrium left atrium of patients with heart failure (β=0.13±0.06, P_{adj}=0.007). An increased capillary size was observed in the left atrium of patients with heart failure (β=0.46±0.22, P_{adj}=0.044). The additional inclusion of 4 PCs into the model did not impact the observed associations between rhythm history, heart failure, or female sex and structural traits of the atrial

Table 1. Patient Characteristics of Donors of LA and RA Samples

	LA (n=75)	RA (n=56)	LA+RA (n=20)	*P* value
Sex, female	25 (33.3)	12 (24.4)	3 (15.0)	0.373
Age, y	63.6±10.4	65.8±10.2	61.5±13.0	0.261
Weight, kg	76.8±14.9	80.1±16.2	79.9±16.8	0.470
Height, cm	173.4±9.6	174.2±10.1	174.8±10.6	0.421
Body mass index	26.0±3.9	26.5±4.2	26.2±3.8	0.524
Rhythm				0.398
NoAF	31 (41.3)	36 (64.3)	9 (45.0)	
pAF	24 (32.0)	13 (23.2)	5 (25.0)	
persAF	20 (26.7)	9 (16.0)	6 (30.0)	
PVI	18 (24.0)	4 (7.1)	2 (10)	0.042
Heart failure	27 (36.0)	14 (25.0)	12 (60.0)	0.045
NYHA class	2.3±1.1	2.4±1.1	2.8±1.1	0.102
HTX	22 (29.3)	7 (12.5)	10 (50.0)	0.007
CHA_2DS_2-VASc	2.9±1.4	2.9±1.5	2.4±1.5	0.227
CAD	32 (42.7)	37 (66.1)	8 (40.0)	0.020
CABG	16 (21.3)	27 (48.2)	4 (20.0)	0.003
Prior MI	18 (24.0)	10 (17.9)	4 (20.0)	0.555
Diabetes	17 (22.7)	10 (17.9)	1 (5.0)	0.349
Chronic kidney failure	8 (10.7)	5 (8.9)	0 (0.0)	0.353
Hypertension	56 (74.6)	48 (85.7)	8 (40.0)	0.001
Prior stroke	10 (13.3)	4 (7.1)	0 (0.0)	0.136
Prior TIA	2 (2.7)	7 (12.5)	1 (5.0)	0.086
Thyroid disease	11 (14.7)	2 (3.6)	4 (20.0)	0.445
MV stenosis	3 (4.0)	3 (5.4)	0 (0.0)	0.013
MV regurgitation				0.065
Grade 1	13 (17.3)	11 (19.6)	5 (25.0)	
Grade 2	12 (16.0)	8 (14.3)	2 (10.0)	
Grade 3	9 (12.0)	7 (12.5)	2 (10.0)	
Grade 4	8 (10.7)	1 (1.8)	5 (25.0)	
MV surgery	19 (25.3)	7 (12.5)	7 (35.0)	0.114
AV stenosis	15 (20.0)	19 (33.9)	1 (5.0)	0.001
AV regurgitation	18 (24.0)	17 (30.3)	4 (20.0)	0.092
AV surgery	18 (24.0)	20 (35.8)	1 (5.00)	0.014
TV regurgitation	23 (30.7)	11 (19.6)	7 (35.0)	0.427

Values are presented as mean±SD for continuous traits or in absolute number (percentage) for dichotomous traits. *P* values were calculated applying 1-way ANOVA or chi-square testing. AV indicates aortic valve; CABG, coronary artery bypass graft; CAD, coronary artery disease; HTX, heart transplant xenograft; LA, left atrial; MI, myocardial infarction; MV, mitral valve; NoAF, no history of AF; NYHA, New York Heart Association; pAF, paroxysmal AF; persAF, persistent/permanent AF; PVI, concomitant pulmonary vein isolation; RA, right atrial; TIA, transient ischemic attack; and TV, tricuspid valve.

myocardium, nor were any of the PCs associated with structural traits (Figure S1; Tables S7 through S8).

After exclusion of younger patients with end-stage heart failure receiving an HTX, age was associated with increased total ECM ratio (LA: β=0.58±0.24, P_{adj}=0.047) as well as with enhanced endomysial fibrosis (LA: 0.13±0.06, P_{adj}=0.036; RA: β=0.18±0.11, P_{adj}=0.034). Capillary density was no longer increased in patients with heart failure after exclusion of patients undergoing HTX (Tables S9 through S16).

A moderate (Pearson) correlation was observed between endomysial fibrosis and total ECM in the LA (r=0.68, P_{adj}<0.001) and RA (r=0.60, P_{adj}<0.001) samples (Figure 5).

Clustering of Left and Right Atrial Samples on the Basis of Structural Features

Unsupervised fuzzy k-means clustering was performed on left (n=91) and right (n=74) atrial samples to identify unique clusters on the basis of fibrotic content (total ECM, endomysial fibrosis, and fibroblast-specific vimentin expression), cardiomyocyte size, and capillary

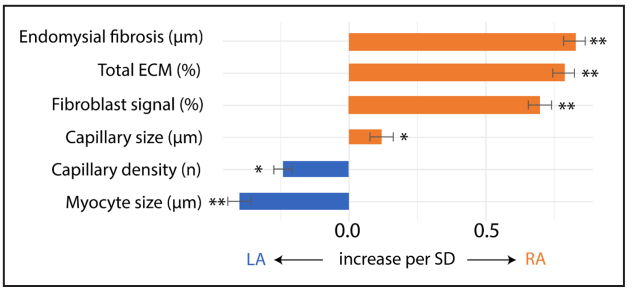

Figure 3. Differences in atrial histology between left atrial (LA) and right atrial (RA) samples.
The association of tissue sampling location (LA/RA) and histological parameters was tested in multivariate models including the covariates: rhythm, heart failure, age, and sex. For visualization, dependent variables were scaled. Dependent variables were standardized to mean 0 and SD of 1. The standardized effect size ($\beta_{standardized}$) was plotted, representing the number of standard deviations change in the dependent variable in the left atrium compared with right atrium. To reduce type I error inflation, permuted *P* values were calculated. *Permuted *P* value <0.05, **Permuted *P* value <0.01. LA/RA differences were confirmed, with more endomysial fibrosis ($\beta_{unstandardized}$=+1.51±0.25 µm, P_{adj}<0.001), a larger total extracellular matrix (ECM) content ($\beta_{unstandardized}$=+6.15±1.14%, P_{adj}0.001), lower capillary density ($\beta_{unstandardized}$=−0.06±0.03 capillaries/myocyte, P_{adj}=0.039), larger capillary size ($\beta_{unstandardized}$=+0.26±0.12 µm, P_{adj}=0.032), but smaller cardiomyocytes ($\beta_{unstandardized}$=−0.92±0.31 µm, P_{adj}=0.005) in the right atrium.

density and size. Due to incomplete phenome data, 4 LA and 2 RA samples were excluded. Following unsupervised identification of the optimal cluster numbers (2 clusters per side), inspection of the within-group sum of squares plot and Silhouette curve confirmed 2 clusters per atrium (Figure S2). Structural properties of the samples within these clusters (Figure 6) suggest that, in each atrium, one cluster contains predominantly samples with more fibrotic features (hereafter named fibrotic atCM; LA: n=22, RA: n=45), while the other cluster contains samples with larger cardiomyocytes (hereafter named hypertrophic atCM; LA: n=69, RA: n=29). LA samples grouped in the fibrotic cardiomyopathy cluster contained more total ECM content (P_{adj}<0.001), endomysial fibrosis (P_{adj}<0.001), and fibroblast-specific vimentin signal (P_{adj}<0.001) and a higher capillary density (P_{adj}<0.001) than samples in the hypertrophic cluster, which contained samples with larger cardiomyocytes (P_{adj}=0.010). Capillary size did not differ. RA samples in the fibrotic cardiomyopathy cluster were characterized by more total ECM (P_{adj}<0.001), more endomysial fibrosis (P_{adj}<0.001), smaller cardiomyocytes (*P*<0.001), and a lower capillary density (P_{adj}=0.013) than samples in the hypertrophic cluster. Fibroblast-specific vimentin signal and capillary size did not differ in the RA clusters (Figure 6; Table S17).

Univariate analysis indicated that patients with fibrotic atCM in the left atrium more often had a history of AF (P_{adj}=0.015) or heart failure (P_{adj}=0.008). Heart failure with moderately or severely reduced left ventricular ejection fraction (P_{adj}=0.046), in particular, was more common in this cluster (Table S18). Moreover, the primary indication for surgery differed between patients showing fibrotic and hypertrophic atCM in the left atrium (P_{adj}=0.034), with more aortic valve surgery in patients with hypertrophic cardiomyopathy, but more HTX in patients with fibrotic cardiomyopathy (Table S18). Patients with fibrotic remodeling in the RA were more often women (P_{adj}<0.001).

Logistic regression in LA samples revealed that, compared with hypertrophic atCM, patients with fibrotic atCM were more often women (OR, 1.33 [95% CI, 1.17–1.47]; P_{adj}=0.002), and had a history of persistent AF (OR, 1.22 [95% CI, 1.02–1.42]; P_{adj}=0.036) or heart failure (OR, 1.62 [95% CI, 1.44–1.78]; P_{adj}<0.001). Patients with fibrotic atCM in the RA were also more frequently female (OR, 1.54 [95% CI, 1.28–1.80]; P_{adj}=0.004) but did not differ in history of persistent AF (OR, 1.06 [95% CI, 0.76–1.36]; P_{adj}=0.655) or heart failure (OR, 0.88 [95% CI, 0.60–1.02]; P_{adj}=0.213). Patients with hypertrophic atCM in the RA were more often male (OR, 1.54 [95% CI, 1.28–1.80]; P_{adj}=0.004; Figure 7).

DISCUSSION

The aim of this study was to better understand the relationship between the main AF-related comorbidities and clinical traits and histological features of atCM in RA and LA tissue samples. We investigated various hallmarks of atCM, such as endomysial and overall fibrosis, fibroblast density, capillary density and size, and cardiomyocyte size in a large European cohort of patients with a variety of indications for cardiac surgery.

The main novelty of the study lies in the systematic analysis of the associations between relevant clinical traits and key histological features of atCM in both right and left atria. We demonstrate strong differences in RA and LA histology and identify heart failure, history of AF, and female sex as the main drivers of fibrosis in the atria. Interestingly, endomysial fibrosis was associated with AF, whereas total ECM content was not, highlighting the relevance of endomysial fibrosis. In addition, there was an association between LA endomysial fibrosis and female sex. Moreover, we identified 2 distinct clusters of samples, 1 showing fibrotic cardiomyopathy and 1 demonstrating a hypertrophic phenotype. Fibrotic cardiomyopathy was associated with female sex, persistent AF, and heart failure, while hypertrophic cardiomyopathy more often occurred in men and in patients undergoing aortic valve surgery. Our findings provide a basis for a better understanding of the association between atCM and cardiovascular diseases.

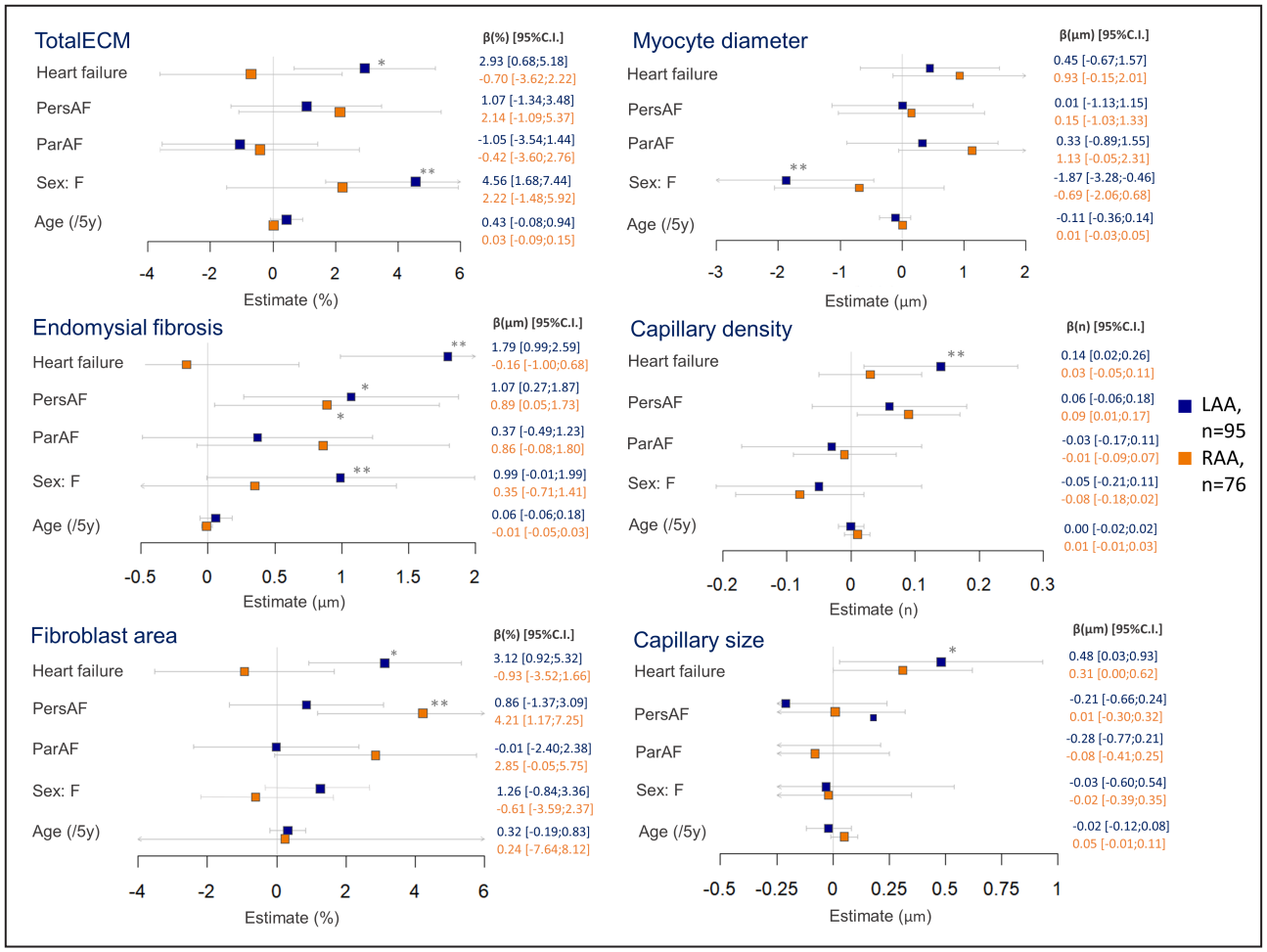

Figure 4. Results of multivariate models describing the association between clinical traits and histological features. Regression models were designed for each histological trait, stratified by atrial side (left atrial [LA], n=95; right atrial [RA], n=76). Sex, heart failure, rhythm history, and age were modeled explicitly as covariates. Overall, heart failure, rhythm history, and sex are the strongest drivers of structural remodeling in this data set. Clinical traits have a slightly more pronounced effect on histology in the left atrium than right atrium. *Permuted *P* value <0.05, **Permuted *P* value <0.01. ECM indicates exctracellular matrix content; LAA, left atrial appendage; ParAF, paroxysmal atrial fibrillation; PersAF, persistent atrial fibrillation; and RAA, right atrial appendage.

Differences in Myocardial Structure in the Left Atrium Versus Right Atrium

We observed more total ECM and endomysial fibrosis, lower capillary density, as well as larger capillaries and smaller cardiomyocytes, in RA samples compared with LA samples. In most published studies, histological assessment of the atrial myocardium is limited to either right atrium or left atrium. As a result, literature on direct right–left comparisons of atrial structural properties in patients is sparse. Our observation of a higher level of fibrosis in the right atrium is in line with an earlier report based on immunohistochemical observations.[36] A postmortem analysis by Platonov et al[37] extensively studied atrial samples from 5 locations in the right and left atrium (superior and inferior pulmonary veins, posterior LA wall, terminal crest, and Bachmann's bundle) and did not observe any difference in fibrotic content, capillary density, or cardiomyocyte size between RA and LA locations. A recent late gadolinium-enhancement cardiac magnetic resonance imaging (LGE-MRI) study showed similar fibrotic levels in right and left atria and a strong correlation between them.[38] At this point, the mechanistic reason for the high fibrotic levels in RA samples reported here remains unknown.

Correlation Between Endomysial Fibrosis and Total ECM Content

Prior research by our group showed that endomysial fibrosis, but not total ECM content, correlates well with AF complexity, that is, the degree of conduction block in fibrillating atria.[23] In the present study, the correlation between total ECM and endomysial fibrosis was significant but only moderately strong. This suggests that to assess the contribution of atrial fibrosis to the electrophysiological substrate for AF, determining

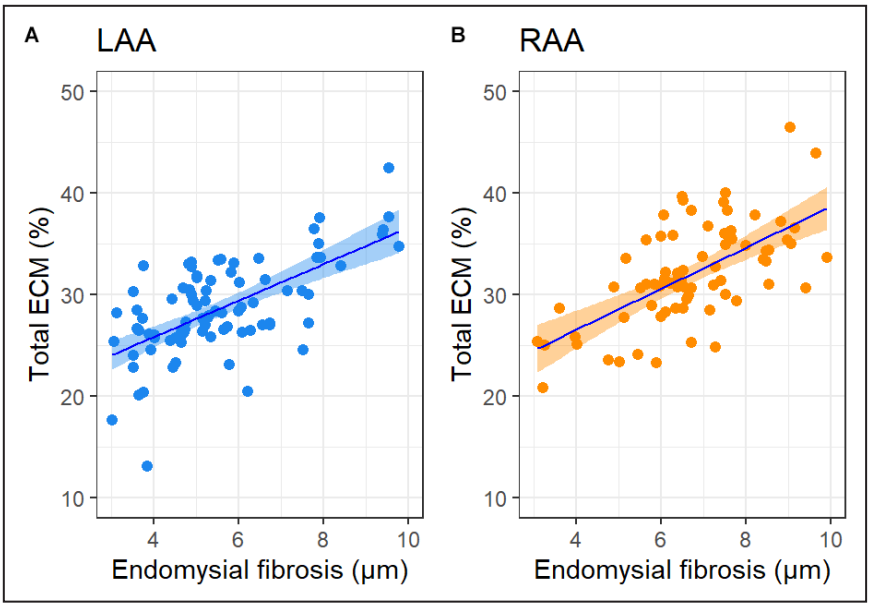

Figure 5. Correlation between total ECM and endomysial fibrosis in LA and RA samples.
Endomysial fibrosis correlates moderately with total ECM (LAA: r=0.68, P_{adj}<0.001; RAA: r=0.60, P_{adj}<0.001). ECM indicates extracellular matrix content; LAA, left atrial appendage; and RAA, right atrial appendage.

endomysial fibrosis may be more relevant than total ECM content quantification. In addition, it is unknown whether LGE-MRI can accurately quantify endomysial fibrosis; it relies on regional differences in a shift in T1 time of the gadolinium-based contrast agent, which may be only sensitive enough to detect thick strands of connective tissue.[39–41] To a substantial extent, those large strands of connective tissue form the physiological skeleton of the atrium and do not necessarily contribute to the electrophysiological substrate for AF. Indeed, the contribution of the diffuse and thin layers of endomyisal fibrosis to the LGE-MRI signal is likely to be minimal. This stresses the need for identifying other markers for endomysial fibrosis, such as noninvasive electrophysiological characteristics or circulating biomolecules.

Heart Failure and Structural Remodeling

Our data confirm previous observations that heart failure is a strong determinant of structural alterations in the atria with an increase in total ECM content, endomysial fibrosis, fibroblast-specific signal, and capillary density and size. Furthermore, these data suggest that heart failure–associated structural changes are more prominent in the left atrium than in the right atrium, most likely as a consequence of chronically increased atrial wall stress caused by pressure or volume overload,[21] which, in most patients, is limited to the left atrium. This hypothesis is supported by the notion of localized conduction heterogeneities due to increased LA fibrosis in dogs or sheep with tachypacing-induced heart failure.[17,22] Additionally, we report more fibroblast-specific vimentin staining in LA samples of heart failure patients. Fibroblasts not only produce ECM, causing fibrosis, but can also couple with myocytes potentially promoting reentry and ectopic activity.[25,42–45]

We observed larger capillary density (left atrium) in patients with heart failure. Pathological pressure or volume overload stretches cardiomyocytes, activating prohypertrophic and proangiogenic signaling proteins to cope with increased oxygen demands.[46–48] Consequently, the observed capillary surplus in the atrial myocardium of patients with heart failure described here could be indicative of an angiogenic response. After exclusion of patients receiving a transplant, capillary density was not altered in patients with heart failure. Patients with AF and heart failure with preserved ejection fraction often show decreased myocardial capillary density,[49,50] resulting in a lower coronary flow reserve and a stiffer myocardium, which are important contributors to diastolic dysfunction.[29,51–54]

AF and Structural Remodeling

Our data showed an association between AF and endomysial fibrosis in human left atrium and right atrium, while AF was not associated with total ECM, altered myocyte size, capillary density, or fibroblast signal. Structural remodeling of atrial myocardium was assessed in animal models of AF by rapid atrial pacing[9–14] and of congestive heart failure by ventricular

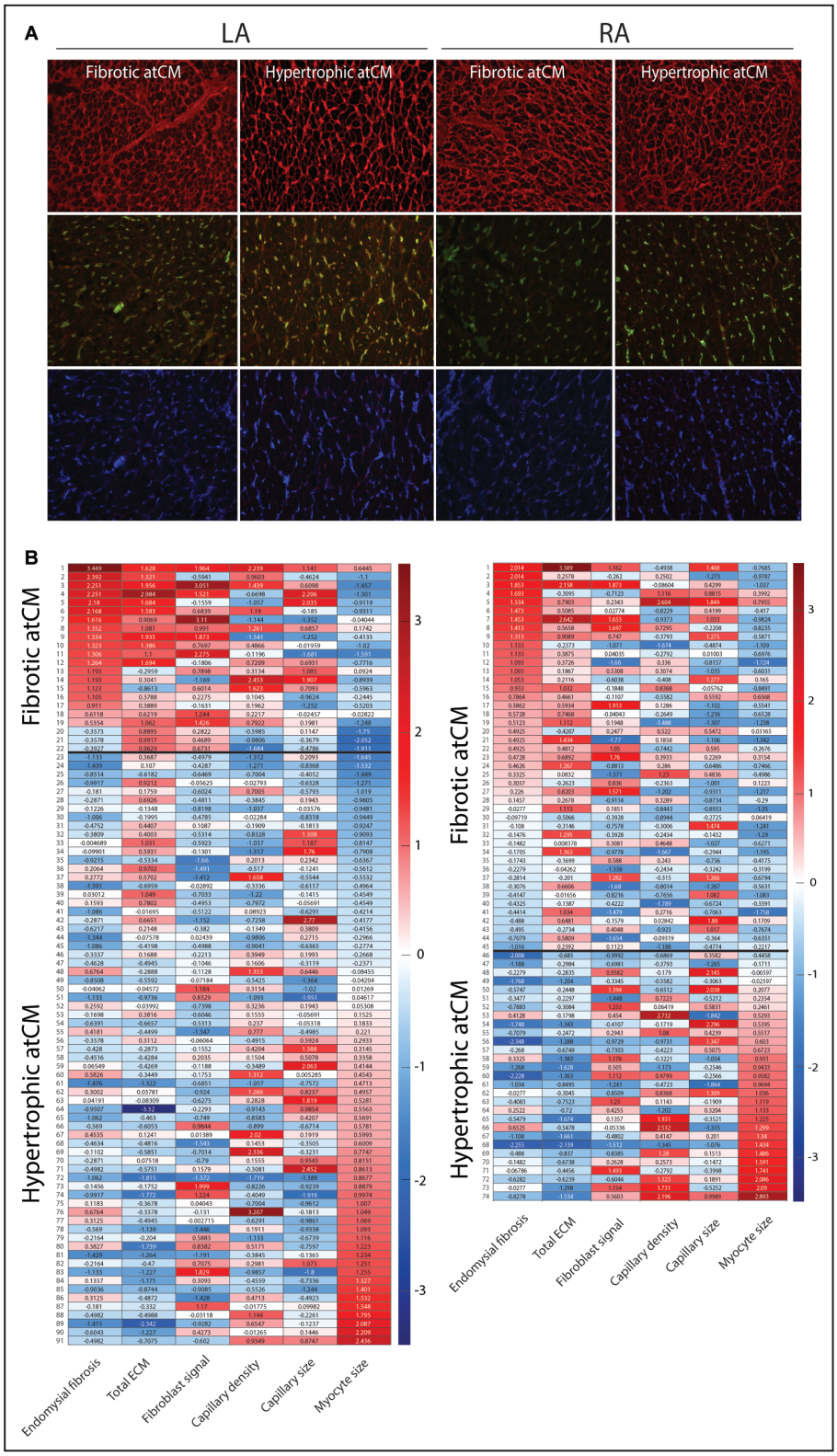

Figure 6. Clustering of LA and RA samples based on structural features.
A, Histological images of tissue features following staining with wheat germ agglutinin (WGA, red), CD31 (green), and vimentin (blue). **B**, Fuzzy k-means clustering was applied to cluster LA and RA samples on structural features. One cluster shows predominantly more fibrotic features, whereas the other cluster contains mainly samples with more pronounced cardiomyocyte hypertrophy. The clusters where therefore named fibrotic atrial cardiomyopathy (atCM) and hypertrophic atrial cardiomyopathy, respectively. ECM indicates extracellular matrix content; LA, left atrial; and RA, right atrial.

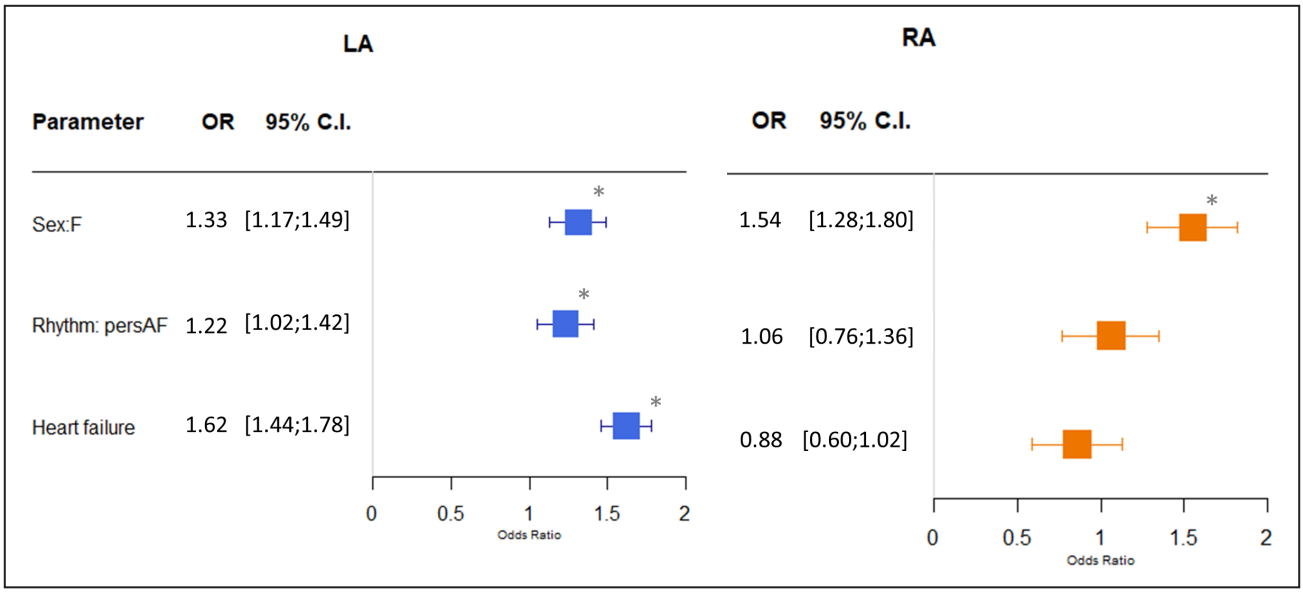

Figure 7. Distribution of clinical traits in fibrotic cardiomyopathy compared with hypertrophic cardiomyopathy.
Patients with fibrotic cardiomyopathy in the left atrium were more often female and more frequently had a history of persAF or heart failure. Fibrotic cardiomyopathy in the right atrium was more frequent in female patients. OR, indicates odds ratio; and persAF, persistent atrial fibrillation. *Permuted *P* value <0.05. Error bars represent SE.

tachypacing.[15–17,55] Ventricular tachypacing models are characterized by rapid, pronounced development of atrial fibrosis, whereas rapid atrial pacing models initially show less pronounced fibrosis.[17,56,57] Fibrosis in ventricular tachypacing models is typified by thick, extended strands of collagen fibers occurring after an initial phase of cardiomyocyte apoptosis,[17,58] indicative of replacement fibrosis. In goats, after 6 months of rapid atrial pacing–induced AF, atrial endomysial fibrosis increased, whereas quantities of total ECM remained constant,[9] a combination of findings corresponding well to the findings of the current study.

Recently, a study of patients undergoing cardiac surgery reported no association between electrophysiological parameters and histological fibrosis markers.[59] Electrophysiological measurements, however, were performed at low rates, and a distinction between total and endomysial fibrosis was made in only a small percentage of patients. Maesen et al[23] demonstrated that among several structural alterations in the atria, endomysial fibrosis, but not total ECM, is the strongest determinant of the degree of conduction block and AF complexity.

From the association between atrial endomysial fibrosis and AF observed here, we cannot infer causality. Structural remodeling, and endomysial fibrosis in particular, was described as a consequence of AF in experimental studies.[9,10,20,60] On the other hand, several studies highlighted the role of both longitudinal[61] and transverse electrical uncoupling resulting from endomysial fibrosis in discontinuous conduction, microreentry,[9,54,62,63] and local heterogeneities of conduction.[9,12,17,23,64] Persistent AF may, therefore, be both a cause and a consequence of endomysial fibrosis.

Aging and Atrial Structural Remodeling

While Gramley et al[65,66] reported an increase in replacement fibrosis with increasing age in RA samples from patients undergoing cardiac surgery, an extensive study by Platonov et al[37] reported no correlation between age and fibrosis in postmortem human atrial samples obtained from the superior and inferior pulmonary veins, posterior LA wall, terminal crest, and Bachmann's bundle. By contrast, both researchers reported a correlation between fibrosis and AF persistence. In their investigations, neither Gramley nor Platonov measured endomysial fibrosis, but quantified total connective tissue content. In the present study, an association between age and endomysial fibrosis was observed in patients with heart failure who did not receive a transplant. Electrical uncoupling of cardiomyocytes,[67] enhanced anisotropy of conduction velocity,[62] and increased fibrosis[68,69] were observed as a result of aging in dogs. Spach et al[70,71] found local slowing of transverse, but not longitudinal, conduction in atrial muscle bundles of elderly patients in whom microfibrosis was more pronounced compared with younger patients. Our data and the referenced experimental data suggest that endomysial fibrosis might contribute to the association between AF and age to some degree. In the present study, however, the contribution of age was limited. A 5-year age difference, causing a 1.5-fold increase in AF prevalence in the general population,[72]

was associated with an 0.06-μm increase in endomysial fibrosis in our study (comparable to 3.3% or 5.6% of the effect of heart failure or persistent AF).

Sex Difference in Atrial Fibrosis

In patients with AF, female sex is associated with a higher risk of stroke,[73] higher recurrence rates following ablation,[74] and increased death,[75] but the underlying pathophysiological mechanisms remain elusive. Several LGE-MRI studies demonstrated an independent association of female sex and atrial fibrosis,[76–78] but it is unclear whether LGE-MRI can distinguish between different forms of fibrosis. There is only limited light microscopy data available on sex differences in fibrosis in atrial tissue. In patients undergoing mitral valve surgery, increased fibrosis in the pulmonary vein sleeves was observed in female patients with AF compared with those without AF, while such a difference was not found in men.[79] Our study is the first to demonstrate an association between female sex and both LA total ECM and endomysial fibrosis. Interestingly, the effect size was comparable to that of persistent AF or heart failure.

Fibrotic Versus Hypertrophic Cardiomyopathy

Clustering methods suggested distinct types of atrial structural remodeling in our cohort: fibrotic and hypertrophic atCM. Patients with fibrotic atCM were more often women and were more likely to have persistent AF or heart failure, while patients with hypertrophic atCM were more often men or patients undergoing aortic valve surgery. This result can be seen as a first step toward the development of a classification of atCM based on histological features and clinical traits associated with them. To a certain degree, our results support aspects of the European Heart Rhythm Association's classification proposed in the consensus paper in 2016.[1] Our data confirm the association between fibrotic alterations and AF or heart failure, while other aspects identified by our study, such as sex differences in endomysial fibrosis and cardiomyocyte size, were not included in the European Heart Rhythm Association classification.[1]

Our study also stresses the need for high-throughput, standardized, automated histological analysis of RA and LA samples. Given the diversity of comorbidities that impact atCM, only dedicated, large-scale, multicenter studies using validated high-throughput histological quantification methods allow full confirmation or refinement of the European Heart Rhythm Association classification of atCM and the assessment of its value in guiding clinical management of AF. The long-term vision remains that a more precise, ideally mechanistic, classification of atrial pathologies may contribute to the development of individualized therapeutic approaches to AF, potentially improving outcomes.

Limitations

Data regarding preoperative medication or nicotine and alcohol consumption were, unfortunately, not complete, and confounding of our data by these factors cannot be ruled out. To minimize heterogeneity between tissue samples in this multicenter cohort, we focused the histological analysis on the myocardial parts of the atrial samples. Additionally, other relevant aspects of atrial architecture, including fatty infiltration, inflammation, or amyloidosis, including endo-epicardial differences in histology, were not studied because of logistical or technical reasons. Moreover, the arrhythmogenic substrate often originates in the atrial sleeves of the pulmonary veins and the posterior wall of the left atrium, but tissue from these regions were not available. Histological properties of atrial appendages might not be fully representative of structural changes in other areas of the atria.

ARTICLE INFORMATION

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Disclosures

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Supplemental Material

Tables S1–S18
Figures S1–S2

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