Extracellular matrix remodeling precedes atrial fibrillation: Results of the PREDICT-AF trial

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BACKGROUND To which extent atrial remodeling occurs before atrial fibrillation (AF) is unknown.

OBJECTIVE The PREventive left atrial appendage resection for the predICTION of fuTure Atrial Fibrillation (PREDICT-AF) study investigated such subclinical remodeling, which may be used for risk stratification and AF prevention.

METHODS Patients (N = 150) without a history of AF with a CHA2DS2-Vasc score of ≥2 at an increased risk of developing AF were included. The left atrial appendage was excised and blood samples were collected during elective cardiothoracic surgery for biomarker discovery. Participants were followed for 2 years with Holter monitoring to determine any atrial tachyarrhythmia after a 50-day blanking period.

RESULTS Eighteen patients (12%) developed incident AF, which was associated with increased tissue gene expression of collagen I (COL1A1), collagen III (COL3A1), and collagen VIII (COL8A2), tenascin-C (TNC), thrombospondin-2 (THBS2), and biglycan (BGN).

Furthermore, the fibroblast activating endothelin-1 (EDN1) and sodium voltage-gated channel beta subunit 2 (SCN2B) were associated with incident AF whereas the Kir2.1 channel (KCNJ2) tended to downregulate. The plasma levels of COL8A2 and TNC correlated with tissue expression and predicted incident AF. A gene panel including tissue KCNJ2, COL1A1, COL8A2, and EDN1 outperformed clinical prediction models in discriminating incident AF.

CONCLUSION The PREDICT-AF study demonstrates that atrial remodeling occurs long before incident AF and implies future potential for early patient identification and therapies to prevent AF (ClinicalTrials.gov identifier NCT03130985).

KEYWORDS Atrial fibrillation; Atrial remodeling; Biomarkers; Collagen VIII; Extracellular matrix; Tenascin

Introduction

The prevalence and incidence of atrial fibrillation (AF) continue to increase. AF is associated with a markedly increased risk of stroke, a diminished quality of life, and a doubled mortality rate. These risks can in part be mitigated, but existing treatments are only modestly effective. It can be postulated that preventing AF itself may prevent or reduce these complications.

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There is ample evidence showing that left atrial fibrosis forms an important substrate for AF. Electrical remodeling forms another important component of atrial remodeling. AF may itself induce electrical and structural remodeling of the atrium, referred to as “AF begets AF.” For neither electrical nor structural remodeling, it is known to which extent it precedes the first onset of AF in patients. Available data are restricted to the use of tissues from patients with prevalent AF or from animal models.

The prospective PREventive left atrial appendage resection for the predIction of fuTure Atrial Fibrillation (PREDICT-AF) study was undertaken to investigate subclinical remodeling preceding AF in the left atrium of patients with no evidence of prior AF but with an increased risk of developing future AF. Patients undergoing cardiac surgery were included, which allowed the collection of left atrial tissues. Patients were rigorously followed for 2 years to determine incident AF occurring ≥50 days after surgery. Left atrial tissue gene expression and histology were associated with incident AF. We further investigated whether the proteins of differentially expressed genes could be detected in circulating blood.

**Methods**

**Clinical study design**

The PREDICT-AF study prospectively enrolled 150 patients with a CHA2DS2-VASc score of ≥2 scheduled for cardiac surgery between May 2015 and June 2018 at the Amsterdam University Medical Centers, University of Amsterdam and the St. Antonius Ziekenhuis, Nieuwegein. The PREDICT-AF study design is summarized below and in Figure 1. Considerations regarding the sample size and a detailed description of the study design and study definitions have been described previously.

All consecutive patients undergoing coronary artery bypass grafting (CABG) or valve surgery in the Amsterdam University Medical Centers or St. Antonius Ziekenhuis, Nieuwegein, were screened for eligibility. A history of AF was ruled out after careful history taking and review of patient’s records and all available rhythm recordings. Inclusion and exclusion criteria are summarized in Online Supplementary Table 1.

During cardiac surgery (baseline), the left atrial appendage was excised and blood samples were collected. Tissue and blood were used for biomarker discovery. Processing is described in Online Supplementary Materials.

The primary outcome was incident AF within 2 years of surgery. Heart rhythm was continuously monitored during the intensive care unit stay followed by regular and symptom-driven electrocardiographic (ECG) recordings until discharge. Patients were followed with outpatient clinic visits after 1, 6, 12, and 24 months (Figure 1). All visits were preceded by 24-hour Holter monitoring and ECG recordings. All information from interval visits, including rhythm recordings and prescribed medication, was collected. All records and rhythm recordings were adjudicated by an independent cardiac electrophysiologist.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the Amsterdam University Medical Centers (Dutch clinical trial registry identifier: NL50754.018.14; ClinicalTrials.gov identifier NCT03130985). All patients provided written informed consent.

**Study outcome**

The primary outcome was incident AF (≥50 days after surgery) within 2 years of follow-up, defined according to the Heart Rhythm Society/European Heart Rhythm Association/European Cardiac Arrhythmia Society consensus statement (≥30-second atrial tachyarrhythmia on Holter monitoring or device registration, or atrial tachyarrhythmia on ECG). The secondary outcome was postoperative AF (POAF), defined as AF within the first 50 days of surgery. A sensitivity analysis for incident AF was performed using a 30-day cutoff.

**Gene selection for primary outcome analysis**

The expression of 26 selected genes was quantified with quantitative real-time polymerase chain reaction in the atrial tissues from all 150 patients. To aid in the selection of candidate genes, RNA sequencing was performed using 22 atrial samples representative of the study cohort (5 incident AF [Online Supplemental Table 2 and Online Supplementary Materials]). Genes were selected on the basis of differential expression and functional relevance implied by gene set enrichment analysis. As RNA sequencing may not have uncovered all relevant gene expression changes, we also considered gene function and complemented the list with the established genes of electrical and structural remodeling (Online Supplemental Tables 3 and 4).

**Statistical analysis**

Categorical and continuous variables were compared using the Fisher exact tests and independent sample t tests or Mann-Whitney U tests, when appropriate.

Missing values were imputed using Multivariate Imputation by Chained Equations (R package “mice”; Online Supplementary Materials). Biomarkers in tissue and blood were correlated using Pearson R correlations. Blood and tissue biomarker levels were centralized to the mean and scaled to the SD. Using Cox proportional hazard models, all variables were assessed for their association with incident AF. False discovery rate–adjusted Q values were calculated for quantitative real-time polymerase chain reaction tissue gene discovery (Benjamini-Hochberg, $\alpha = 0.2$). Variables were selected on the basis of a (nonadjusted) entry criterion of $P < .1$ for forward selection. Biomarkers were assessed using bootstrapped and imputed multivariable models. Hazard ratios (HRs), 95% confidence intervals, and C statistics were calculated.

The discriminative values (C statistics) of a panel of tissue or plasma biomarkers and those of clinical biomarkers only, including established risk factors such as age ≥75 years, female sex, and left atrial volume index (LAVI),
were assessed. All statistical analyses were performed using R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

Data availability
The transcriptome sequencing data are available under EGAS00001005295.

Results
Patient characteristics
Of the 1941 patients who underwent cardiothoracic surgery screened for participation, 528 (27.2%) were eligible and approached for study participation. Of these, 150 patients provided informed consent and were included (Figure 1). Patient characteristics are summarized in Table 1. One hundred fifteen patients (77%) underwent CABG, 11 (7%) underwent

![Figure 1](image-url)
Table 1  Patient characteristics (N = 150)

| Characteristic                        | Value                  |
|---------------------------------------|------------------------|
| Age (y)                               | 68.1 ± 7.4             |
| Sex: female                           | 19 (12.7)              |
| Body mass index (kg/m²)               | 28.0 ± 3.5             |
| Left atrial volume index (mL/m²)      | 30.6 ± 9.5             |
| Left ventricular ejection fraction (%)| 47.9 ± 11              |
| CABG only                             | 115 (76.7)             |
| Valve surgery only                    | 11 (7.3)               |
| CABG + valve surgery                  | 24 (16.0)              |
| Myocardial infarction                 | 46 (30.7)              |
| Percutaneous coronary intervention    | 36 (24.0)              |
| Chronic obstructive pulmonary disease | 6 (4.0)                |
| Hypertension                          | 95 (63.3)              |
| Diabetes mellitus                     | 47 (31.3)              |
| Congestive heart failure              | 0 (0)                  |
| Stroke/transient ischemic attack/embolus | 17 (11.3)            |
| CHA2DS2-VASc score ≥4                | 49 (32.7)              |
| Creatinine (µmol/L)                   | 90.0 ± 20.2            |
| Hemoglobin (mmol/L)                   | 8.9 ± 0.8              |
| Class IA AAD                          | 0 (0)                  |
| Class IC AAD                          | 0 (0)                  |
| Class II AAD                          | 106 (71)               |
| Class III AAD                         | 1 (0.6)*               |
| Class IV AAD                          | 5 (3.3)                |
| Digoxin                               | 0 (0)                  |

Values are presented as mean ± SD, median (interquartile range), or n (%).

AAD = antiarrhythmic drug; CABG = coronary artery bypass grafting.

Suppl. Table 1.

valve surgery, and 24 (16%) underwent combined CABG and valve surgery (Table 1). In all patients, the left atrial appendage was excised (Figure 1).

Primary and secondary outcomes

Patients were followed for 2.0 (1.9–2.0) years (Figure 1). Two patients (1%) died postoperatively, 7 patients (5%) died thereafter, and 13 patients (9%) were lost to follow-up. The primary outcome incident AF occurred in 18 patients (12%) during 2-year follow-up. Fifteen of those (83%) also experienced POAF. The secondary outcome POAF was detected in 63 patients (42%) <50 days post surgery. A 50-day cutoff for POAF was corroborated, as 6 of 8 patients who experienced POAF between 30 and 50 days did not have an AF episode thereafter (Online Supplemental Figure 2).

No clinical risk factors were associated with incident AF (Figure 2A). Established risk factors including age ≥75 years (HR 2.43 [0.91–6.48]; P = .077), LAVI (HR 1.04 [0.99–1.09] per mL/m² increase; P = .052), and N-terminal pro-B-type natriuretic peptide (HR 1.30 [0.95–1.79]; P = .098) showed a nonsignificant increase in patients who develop incident AF (Figure 2A).

POAF was associated with increased LAVI (HR 1.05 [1.02–1.07]; P = .00014) and left ventricular ejection fraction (HR 1.03 [1.00–1.06]; P = .034). Isolated CABG, opposed to (concomitant) valve surgery, decreased the risk of POAF (Figure 2B).

Gene discovery

RNA sequencing discovered type VIII collagen (COL8A2) and fucosyltransferase 4 (FUT4) as the most significant among 453 differentially expressed genes (Figure 3A). The top upregulated biological processes were mostly involved in extracellular matrix (ECM) remodeling including ECM proteoglycans and ECM glycoproteins (Figure 3B and Online Supplemental Figures 3 and 4). ECM regulatory processes such as epithelial to mesenchymal transition, bone morphogenetic protein signaling, and Wnt signaling were also upregulated. Downregulated pathways such as adipogenesis and cellular respiration suggested decreased energy production and metabolism along with downregulated genes such as acyl-CoA oxidase 1 (ACOX1), glycogen phosphorylase muscle associate (PYGM), and cardiolipin synthase 1 (CRLS1).

Matricellular gene expression predicts incident AF

Twenty-six genes were selected for quantitative real-time polymerase chain reaction quantification (Figure 4) on the basis of RNA sequencing or function in electrical or structural remodeling (see details in Online Supplemental Table 3). Increased expression of ECM genes was associated with incident AF, including fibrillar type I and type III collagen (COL1A1; HR 1.42 [1.08–1.87]; P = .013 and COL3A1; HR 1.37 [1.00–1.87]; P = .049) and nonfibrillar type VIII collagen (COL8A2; HR 1.70 [1.16–2.48]; P = .006) (Figure 4). Furthermore, thrombospondin-2 (THBS2; HR 1.67 [1.16–2.41]; P = .006), tenascin-C (TNC; HR 1.45 [1.04–2.01]; P = .027), biglycan (BGN; HR 1.64 [1.03–2.61]; P = .039), and the fibroblast activating factor endothelin-1 (EDN1; HR 1.43 [1.07–1.91]; P = .016) were associated with incident AF.

The regulatory sodium voltage-gated channel β subunit 2 (SNC2B) was the only upregulated electrical remodeling–related gene associated with incident AF (HR 1.99 [1.31–3.00]; P = .001). Decreased expression of the Kir2.1 channel (KCNJ2) was nonsignificantly associated with incident AF (HR 0.60 [0.33–1.08]; P = .087).

A sensitivity analysis using a 30-day cutoff for incident AF showed similar but slightly attenuated results (Online Supplemental Figure 5). None of the quantified gene expression levels were associated with POAF (Online Supplemental Figure 6).

Blood biomarkers

COL8A2, THBS2, and TNC protein levels were determined in patients’ plasma samples. Elevated plasma levels of TNC (HR 1.51 [1.02–2.23]; P = .04) predicted incident AF, and plasma levels of COL8A2 showed a nonsignificant increase in incident AF (HR 1.36 [0.99–1.88]; P = .062) (Figure 5A). POAF was not associated with increased plasma biomarker levels (Online Supplemental Figure 7).
Tissue and plasma TNC levels were weakly but significantly correlated ($R = 0.3; P = .00031$). Tissue and plasma COL8A2 levels did not correlate in all patients ($R = 0.0094; P = .91$), but in the patients with higher than mean COL8A2 tissue expression, the correlation was highly significant ($R = 0.40; P = .0062$) (Figures 5B and 5C).

**Biomarker panel for incident AF**

Gene clustering showed that ECM genes EDN1, THBS2, TNC, fibronectin, COL1A1, and COL3A1 formed a gene cluster. COL1A1 and COL3A1 showed a remarkably strong correlation ($R = 0.95; P = 6.65 \times 10^{-97}$) (Figure 6A).
Forward selection identified BGN (HR 2.03 [1.14–3.28]; P = .008), COL8A2 (HR 2.12 [1.46–3.56]; P < .001), EDN1 (HR 1.70 [1.12–2.86]; P = .052), and KCNJ2 (HR 0.43 [0.18–0.78]; P = .025) as tissue markers associated with incident AF (P < .05) (Figure 6B). The most discriminative combination of ≤4 biomarkers encompassing only clinical risk factors (age ≥ 75 years and LAVI; female sex did not improve the model) yielded an area under the curve of 0.697 [0.553–0.824], which increased to 0.777 [0.674–0.872] with additional plasma COL8A2 and TNC. A panel of 4 tissue biomarkers predicted incident AF best (area under the curve 0.843 [0.759–0.915]) (Figure 6C).

Histological changes preceding incident AF
Interstitial collagen fractions were similar in patients with and without incident AF (Figures 7A and 7B). However, the total collagen fraction including endo- and (sub)epicardium was numerically larger in patients with incident AF than in patients without (51.9% vs 44.1%; P = .091). The total area of fibrotic (sub)epicardium was larger in patients with incident AF (95.2 [72–181] μm²/cardio myocyte vs 35.2 [22–49] μm²/cardiom yocyte; P = .0010). The total area of epicardial vimentin-positive mesenchymal cells, mostly fibroblasts, was also larger (26.3 [16.1–43.6] μm²/cardiom yocyte vs 9.8 [6.4–14.4] μm²/ cardiom yocyte; P = .0034). The interstitial vimentin-positive mesenchymal cell fraction and the CD31-positive cell fraction, a marker for microvessel density, were similar in patients with or without incident AF (Online Supplemental Figure 8).

Discussion
The PREDICT-AF study provides the first evidence for signs of atrial remodeling long before the onset of incident AF in patients. The discovered gene expression changes that associated with incident AF predominantly pertained to increased expression of matricellular genes and proteoglycans. Our data also suggest that expression changes in ion channel genes may occur before incident AF, though less abundantly. Overall, gene expression in the left atrium was more predictive of incident AF than were clinical parameters alone, which underscores the need for noninvasive detection.

ECM gene expression precedes incident AF
Atrial structural remodeling is considered a key determinant of the substrate of AF.4,10 However, based on the available reports, it cannot be determined whether atrial fibrosis is the result of AF-induced remodeling or whether it is part of a preexisting substrate. Atrial fibrosis has been associated with POAF, but prospective studies have not investigated long-term incident AF.11 The lack of an association between ECM gene expression and POAF in this study suggests a distinct pathophysiology of POAF and incident AF. At the same time, the high rate of POAF in patients with incident AF supports the concept that POAF in itself may be an early sign of future substrate changes.11

Case-control studies associated increased ECM deposits with lone AF or AF secondary to mitral valve disease.7,12 In tachy-paced heart failure dogs and several other animal models, atrial fibrosis was associated with AF susceptibility.6 However, animal models remain inherently limited by the artificiality of induced AF.

Here, we confirm the subclinical increase in COL1A1 and COL3A1 messenger RNA as markers of incident AF. Moreover, increased tissue expression of COL8A2, BGN, TNC, and THBS2 was associated with incident AF and neither have previously been described in relation to atrial tissue remodeling in AF. COL8A2 and COL8A1 (both upregulated)
are expressed by cardiac fibroblasts and induce fibroblast proliferation and smooth muscle cell migration.\textsuperscript{13} COL8A2 is elevated in dilated cardiomyopathy.\textsuperscript{14} BGN, associated with fibroblast activation and interacting with collagens, was increased in mice with myocardial infarction (MI).\textsuperscript{15,16} TNC regulates cellular adhesion, cell migration, and myofibroblast differentiation, and expression is increased in heart failure and MI.\textsuperscript{17} THBS2 may regulate matrix metalloproteinase-2 activity and fibroblast adhesion and was increased in MI.\textsuperscript{17,18}

EDN1 could regulate ECM gene expression by acting as a fibroblast activator.\textsuperscript{19} EDN1 can increase sarcoplasmic reticulum Ca\textsuperscript{2+} release and has been associated with atrial dilatation, atrial fibrosis, and AF.\textsuperscript{19,20}

These genes therefore not only have direct structural roles in ECM remodeling but may have various regulatory functions, making them potential targets for preventive therapies.\textsuperscript{17,21}

The interstitial collagen fraction was unchanged in patients with incident AF. The discovered gene expression changes may be early signs of tissue remodeling, and histological structural remodeling may be detectable only later. However, the total collagen and epicardial fibrotic area including mesenchymal cell fraction were increased in patients before incident AF. These early morphological changes in the epicardium suggest that epithelial to mesenchymal transition, the most upregulated biological process in patients with incident AF, may be ongoing. Epicardial reactivation may increase interstitial (myo)fibroblasts numbers and activate ECM remodeling.\textsuperscript{22}

**Metabolic pathways are downregulated**

Downregulated processes in incident AF were related to energy metabolism and adipogenesis. A similar downregulation was reported in prevalent AF. It was suggested that AF causes increased energy demands initiating an uncoordinated response and increased tissue inhomogeneity.\textsuperscript{23} We observed decreased energy metabolism preceding AF, suggesting that this process is not the result of AF itself. Future functional analyses are needed to investigate the underlying mechanisms.

**Early electrical remodeling is subtle**

The lack of an association of ion channel gene expression with incident AF could indicate that electrical remodeling

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### Tissue biomarkers of incident AF

| Extracellular matrix | Hazard Ratio [95% CI] | P-value | FDR | Q-value |
|----------------------|-----------------------|---------|------|---------|
| Collagen 1 (COL1A1)  | 1.42 [1.08–1.87]      | 0.013   | 0.031|
| Collagen 3 (COL3A1)  | 1.37 [1.00–1.87]      | 0.049   | 0.062|
| Collagen 6 (COL6A3)  | 1.10 [0.70–1.73]      | 0.67    | 0.15 |
| Collagen 8 (COL8A2)  | 1.70 [1.16–2.48]      | 0.006   | 0.023|
| Fibronectin (FN1)    | 1.38 [0.90–2.11]      | 0.14    | 0.085|
| Versican (VCAN)      | 1.12 [0.75–1.67]      | 0.58    | 0.13 |
| Biglycan (BGN)       | 1.64 [1.03–2.61]      | 0.039   | 0.054|
| Periostin (POSTN)    | 1.06 [0.69–1.64]      | 0.79    | 0.18 |
| Thrombospondin (THBS2)| 1.68 [1.16–2.41]     | 0.006   | 0.015|
| Tenascin (TNC)       | 1.45 [1.04–2.01]      | 0.027   | 0.046|
| Heparan sulf proteoglyc 2 (HSPG2)| 0.85 [0.52–1.42] | 0.53    | 0.12 |
| Integrin subunit-α10 (ITGA10)| 0.90 [0.54–1.51] | 0.69    | 0.18 |
| Elastin microfibril interf 1 (EMILIN1)| 1.14 [0.75–1.75] | 0.53    | 0.12 |
| Glypican 6 (GPC6)    | 1.06 [0.70–1.62]      | 0.77    | 0.17 |

| Ion Channels | Hazard Ratio [95% CI] | P-value | FDR | Q-value |
|-------------|-----------------------|---------|------|---------|
| Na\textsuperscript{+} Channel β2 unit (SCN2B) | 1.99 [1.31–3.00] | 0.001 | 0.008|
| Kir2.1 K\textsuperscript{+} Channel (KCNJ2) | 0.60 [0.33–1.08] | 0.087 | 0.077|
| Cav3.1 channel (CACNA1G) | 0.89 [0.51–1.57] | 0.69 | 0.15 |
| Cav alpha 2 delta 3 (CACNA2D3) | 0.89 [0.53–1.49] | 0.67 | 0.14 |
| Kv interacting protein 2 (KCNIpII) | 0.81 [0.49–1.35] | 0.43 | 0.11 |
| Nav1.8 channel (SCN10A) | 0.96 [0.60–1.56] | 0.88 | 0.18 |
| Nav1.5 channel (SCN5A) | 0.78 [0.43–1.39] | 0.39 | 0.10 |
| I(Kr) alpha subunit (KCNH2) | 0.97 [0.61–1.55] | 0.90 | 0.19 |
| Kir3.2 channel (KCNJ5) | 1.21 [0.79–1.86] | 0.37 | 0.09 |
| I(Ks) alpha subunit (KCNQ1) | 1.01 [0.63–1.62] | 0.96 | 0.20 |

| Other | Hazard Ratio [95% CI] | P-value | FDR | Q-value |
|-------|-----------------------|---------|------|---------|
| Endothelin-1 (EDN1) | 1.43 [1.07–1.91] | 0.016  | 0.038|
| Fucosyltransferase-4 (FUT4) | 1.42 [0.96–2.09] | 0.078  | 0.069|

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**Figure 4**  Tissue gene expression and incident AF. Upregulation of 6 extracellular matrix genes and EDN1 associated with incident AF. SCN2B was upregulated and KCNJ2 nonsignificantly downregulated. Boldface indicates significant values (false discovery rate adjusted; Benjamini-Hochberg critical value 0.049 < 0.062). AF = atrial fibrillation; CI = confidence interval; EDN1 = endothelin-1; FDR = false discovery rate adjusted; KCNJ2 = Kir2.1 channel; SCN2B = sodium voltage-gated channel β subunit 2.
occurs at the protein or functional level only or that changes occur close to or after AF develops (AF begets AF). Still, we found that SCN2B was increased and KCNJ2 tended to decrease preceding AF. SCN2B is associated with familial AF and AF susceptibility and modulates the sodium current density. KCNJ2 encodes the Kir2.1 channel and is associated with QT shortening, proarrhythmogenic remodeling, and AF.

**Risk stratification and prevention**

Tissue biomarkers are not applicable in clinical practice at this point, but we demonstrated that increased plasma levels of COL8A2 and TNC correlated to tissue expression and predicted incident AF. Several circulating markers of inflammation, heart failure, and growth factors (eg, N-terminal pro B-type natriuretic peptide and fibroblast growth factor 23) have previously been associated with incident AF, but for most established biomarkers it is unresolved whether these reflect ongoing atrial remodeling or a remote process.

Based on their function, COL8A2 and TNC could be novel circulating biomarkers of interest. COL8A2 is expressed in the (sub)endothelium and upregulated with atrial remodeling in mice. Plasma TNC levels are increased in patients with prevalent AF and have prognostic value in patients with heart failure or MI.

Plasma COL8A2 and TNC levels alone demonstrated insufficient sensitivity or specificity for identifying at-risk patients. The correlations between tissue and plasma of COL8A2 and TNC were rather weak, and THBS2 was not

**Figure 5** Plasma biomarkers and incident AF. A: Plasma TNC levels associated with incident AF and plasma COL8A2 levels were nonsignificantly elevated in patients with incident AF. B: Left panel: Correlation between TNC tissue and TNC plasma levels. Right panel: Correlation after exclusion of 1 extreme value. C: COL8A2 tissue and COL8A2 plasma levels did not correlate (left), though a significant correlation is found in patients with tissue expression levels above the mean (right). AF = atrial fibrillation; CI = confidence interval; COL8A2 = type VIII collagen; expr = expression; TNC = tenascin-C.
correlated with tissue levels or predictive of incident AF. Discrepancies between tissue and blood may result from remote excretion or differences in marker excretion or degradation. Nevertheless, adding plasma TNC and COL8A2 levels to clinical markers in a risk model increased the predictive value for incident AF as compared with clinical factors alone. Together, the data provide proof of principle that circulating markers, combined with clinical markers such as LAVI, can refine the identification of patients at risk of AF while also providing information on subclinical atrial remodeling.

**Figure 6** ECM gene correlations and multivariable analysis. A: Correlation matrix of tissue gene expression. Only significant values ($P < .05$) are depicted. Hierarchical clustering identified a cluster of ECM genes. B: Forward selection identified $BGN$, $COL8A2$, $EDN1$, and $KCNJ2$ as predictors of incident AF. C: $C$ statistics (95% confidence interval) of a combination of clinical factors, clinical factors with plasma biomarkers, or a panel of any biomarker for discriminating incident AF. AF = atrial fibrillation; $BGN$ = biglycan; CI = confidence interval; $COL8A2$ = type VIII collagen; $EDN1$ = endothelin-1; $KCNJ2$ = Kir2.1 channel; $LAVI$ = left atrial volume index; NT-proBNP = N-terminal pro-B-type natriuretic peptide; TNC = tenascin-C.
Future studies could refine the discriminative value of biomarkers by investigating the change in biomarker levels combined with the development of clinical risk factors over time.

Limitations

The unique study design pertaining to the biomaterial collection of patients without prevalent disease in a prospective study has inherent limitations. First, the limited sample size precluded subgroup analysis and might have prevented clinical parameters such as LAVI and age from reaching statistical significance. Nevertheless, the relative importance of a gene panel and blood biomarkers compared with these clinical parameters indicates the relevance of tissue remodeling compared with clinical factors in predicting incident AF.

Second, for ethical reasons the study population was restricted to a population of patients who underwent cardiothoracic surgery, consisting mostly of male patients who underwent CABG, and with an indication for anticoagulation therapy (CHA2DS2-VASc scores ≥ 2) in case incident AF would develop.8 As a result, all patients had preexisting cardiac disease and possibly atrial remodeling. It is possible that the present results are relevant for patients with vascular disease only. Nevertheless, included patients have comorbidities that are common in a general population with AF2 and the consistency of our results with the established mechanisms of atrial remodeling makes our findings likely relevant to a broader population of patients at risk of AF.

The distinction between POAF and incident AF can be challenging and despite consensus on a 30-day cutoff for POAF, there is evidence for POAF recurrences after 30 days postoperatively.33 Here, a 50-day cutoff was used, as clinical and biological data suggested that AF between 30 and 50 days were mostly late POAF recurrence. The use of a 30- or 50-day cutoff did not significantly affect the results.

Patients were monitored with frequent Holter monitoring during follow-up, in accordance with current recommendations.2,9 However, no continuous monitoring with implantable loop recorders was available and some interval AF episodes may have been missed.

Patients were treated according to the standard of care, and participants may have received medication during follow-up that could affect incident AF. However, the number of patients receiving medication such as antiarrhythmic drugs or aldosterone antagonists was low and drugs were not protective against incident AF.
Finally, the prediction models built may be prone to overfitting despite applying statistical techniques such as bootstrapping to minimize these effects. Overall, the study design remains explorative, and results need confirmation in larger populations outside the cardiac surgery setting.

Conclusion

Signs of atrial remodeling, consisting of complex ECM and electrical remodeling, develop long before the first clinical detection of AF. A tissue gene panel was best at discriminating future AF. Circulating blood biomarker levels reflect tissue ECM gene expression. As a result, our findings may direct future research aiming at early patient identification and the discovery of substrate targets for primary prevention.

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Appendix

Supplementary data

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.hrthm.2021.07.059.

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