Left Atrial Transcriptional Changes Associated With Atrial Fibrillation Susceptibility and Persistence

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Background—Prior transcriptional studies of atrial fibrillation (AF) have been limited to specific transcripts, animal models, chronic AF, right atria, or small samples. We sought to characterize the left atrial transcriptome in human AF to distinguish changes related to AF susceptibility and persistence.

Methods and Results—Left atrial appendages from 239 patients stratified by coronary artery disease, valve disease, and AF history (no history of AF, AF history in sinus rhythm at surgery, and AF history in AF at surgery) were selected for genome-wide mRNA microarray profiling. Transcripts were examined for differential expression with AF phenotype group. Enrichment in differentially expressed genes was examined in 3 gene set collections: a transcription factor collection, defined by shared conserved cis-regulatory motifs, a miRNA collection, defined by shared 3′ untranslated region motifs, and a molecular function collection, defined by shared Gene Ontology molecular function. AF susceptibility was associated with decreased expression of the targets of CREB/ATF family, heat-shock factor 1, ATF6, SRF, and E2F1 transcription factors. Persistent AF activity was associated with decreased expression in genes and gene sets related to ion channel function consistent with reported functional changes.

Conclusions—AF susceptibility was associated with decreased expression of targets of several transcription factors related to inflammation, oxidation, and cellular stress responses. In contrast, changes in ion channel expression were associated with AF activity but were limited in AF susceptibility. Our results suggest that significant transcriptional remodeling marks susceptibility to AF, whereas remodeling of ion channel expression occurs later in the progression or as a consequence of AF. (Circ Arrhythm Electrophysiol. 2015;8:32-41. DOI: 10.1161/CIRCEP.114.001632.)

Key Words: ATF transcription ▪ atrial fibrillation ▪ transcriptome

Atrial fibrillation (AF) is the most common clinically encountered arrhythmia, and AF prevalence is projected to rise with the aging population and the increased prevalence of comorbid diseases. AF is associated with significant morbidity and mortality, including a quadrupled risk of heart failure and a nearly doubled risk of death.1 In addition, AF induces electric, structural, and autonomic changes that facilitate AF persistence and recurrence after intervention.2 As such, it is imperative to understand the early and causative changes in AF pathogenesis.

Editorial see p 5

Known risk factors for AF are diverse and include advancing age, hypertension, valvular disease, coronary artery disease (CAD), endocrine disorders, and genetic polymorphisms. Various pathophysiological mechanisms have been implicated in the development of AF;2 Current models involve the interplay of ectopic triggers within pulmonary vein ostia and reentrant circuits within an anatomically and electrically remodeled atrium.1,3 As the insults leading to AF are varied and AF is associated with AF-initiated proarrhythmic changes, the unifying mechanisms that precede chronic forms of AF remain uncertain. Although recent genome-wide association studies have implicated loci near or in embryonically active genes that may promote pulmonary vein susceptibility, it remains unclear why AF usually does not develop until many decades after pulmonary vein development.

Microarray expression analyses of atrial tissues can provide a global unbiased framework to characterize the transcriptional changes associated with AF susceptibility and progression. Initial transcriptional studies of AF focused on restricted sets of candidate transcripts chosen for biological plausibility. These studies associated remodeling in AF with changes in the expression of...
WHAT IS KNOWN

- Significant anatomic and cellular remodeling both precede and are induced by atrial fibrillation (AF); however, previous investigations have been limited to specific transcripts, animal models, chronic AF, right atria, or small samples.
- Several genetic loci have been associated with the development of AF; however, AF usually manifests in advancing age and in the setting of comorbid cardiovascular disease.

WHAT THE STUDY ADDS

- This study provides a genome-wide assessment of the changes in gene expression and regulation that are associated with the development and persistence of AF.
- Several cellular stress mechanisms are associated with susceptibility to development of AF.
- Changes in ion channel expression are more associated with persistence of AF.
- A list of potential upstream targets to prevent or reverse the development of AF beyond current ion channel modulating antiarrhythmic therapies is provided.

ion channels and components of cellular signaling cascades. Moreover, prior transcriptomic, proteomic, and metabolomic investigations of AF have been mostly limited to animal models, right atria, and chronic AF. Although these studies have correlated transcripional and functional changes with many aspects of AF-associated remodeling, it remains undetermined whether the demonstrated changes are commonly present in human left atria, the chamber most associated with AF initiation, and whether they are causative or are caused by AF, and to what degree concomitant cardiovascular diseases contribute.

Approximately 70% to 80% of patients with AF have structural heart disease. With access to a significantly larger sample size than used in prior studies, we sought to characterize alterations of the left atrial transcriptome associated with paroxysmal or nonpersistent AF susceptibility in patients with underlying CAD or valvular disease and to relate these changes to those seen with more advanced, persistent AF.

Methods

Patient and Tissue Characteristics

Atrial tissues of 239 patients were selected from a repository of left atrial appendage tissues obtained from patients who underwent cardiac surgery at the Cleveland Clinic and who consented to have discarded tissue used for research. Left atrial appendage tissues were also obtained from 2 donor hearts without AF that were unsuitable for transplantation. Donor families consented to use the tissues for research. Tissue collection and investigations were performed in accordance with a Cleveland Clinic Institutional Review Board-approved protocol. Samples were stored at −80°C before RNA extraction.

Three AF phenotypes were determined by review of AF history and electrocardiograms taken just before the surgery: No history of AF (NoAF), history of AF but in sinus rhythm at time of surgery (AF/SR), and history of AF in persistent AF or flutter at the time of surgery (AF/AF). The AF/SR samples represented those with susceptibility to AF when compared with NoAF samples, whereas AF/AF compared with AF/SR samples reflected persistent AF activity, and AF/AF samples reflected both AF susceptibility and persistent AF activity when compared with NoAF samples (Figure 1). Patient clinical characteristics are summarized in Table 1.

RNA Preparation, Microarray Testing, and Data Extraction

Total RNA was extracted using the Trizol technique. Samples were assayed using Illumina Human HT-12 mRNA microarrays in the Cleveland Clinic Lerner Research Institute Genomics Core Laboratory. For each sample, 250-ng RNA was reverse transcribed into complementary RNA and biotin-UTP labeled using the TotalPrep RNA Amplification Kit (Ambion, Austin, TX). Complementary RNA was quantified using a Nanodrop spectrophotometer and complementary RNA size distribution was assessed on a 1% agarose gel. Complementary RNA was hybridized to Illumina Human HT-12 Expression BeadChip arrays (v.3/4). Arrays were scanned using a BeadArray reader.

Raw mRNA expression data were extracted using Beadstudio (Illumina). Background correction was conducted by fitting a normal-gamma deconvolution model to the background subtracted signal. Quantile normalization and batch-effect adjustment were performed using the beadarray and ComBat R-packages. Probes that were not detected (at a P value <0.05) in ≥50% of samples were excluded. For genes that mapped to multiple probes, the probe with the highest mean expression level was selected for gene set analysis.

Probe-Level Differential Expression

For each atrial transcript that survived quality control and filtering, linear regression analysis was performed against the AF phenotype adjusting for sex, age, CAD, and mitral valve disease.

Factor analysis, implemented in the R-package FAMT, was used to adjust expression values for additional sources of variation, such as uncontrolled factors in experimental design or unmodeled biological factors, not captured by linear modeling. Factor analysis infers the presence of latent variables directly from the expression data and may be used to correct for biological factors which affect gene expression when not explicitly included in the linear model. Moderated t statistics, implemented in the R-package limma, were used to obtain P values. Genome-wide significance was determined using a false discovery rate <0.05.

The top 50 probes from the 3 differential expression gene lists comparing AF rhythms were chosen for display. Raw expression values of these 50 probes were regressed against the controlling variables (sex, age, CAD, and mitral valve disease). The residuals were aggregated...
toward the medians to generate gene level expression values. Finally, expression values were normalized by dividing by the SD to allow comparison across genes. Heatmaps were generated to illustrate the different expression patterns between AF rhythms using an unsupervised clustering approach.

Gene Set Analysis
A gene set–based analysis was used to further characterize differential expression with AF phenotype using sets of genes known to have common biological significance. These sets of genes were then examined for changes in expression with AF phenotype, as described below. By grouping information from multiple genes, this method allows for identification of significant changes in sets of functionally related genes even when the individual genes that compose a gene set do not demonstrate differential expression that is significant at a genome-wide level.

Specifically, for each comparison of AF phenotype groups, competitive gene set enrichment was implemented in the romer function of limma (100000 rotations, test statistic: mean). Romer was chosen as it is compatible with linear modeling, accounts for the inherent correlation structure of genes (unlike gene permutation), and uses a parametric resampling method for calculating P values that avoids the limitations of comparable permutation methods.10 Gene set collections obtained from the Molecular Signature Databases of the Broad Institute were examined.11 To account for overlap in member transcripts between gene sets in our interpretation, significant gene sets were clustered on the basis of member genes as described in Methods I in the Data Supplement.

Results
Gene-Wise Differential Expression by AF Phenotype
Of the 49576 probes assayed, after filtering, 11806 were expressed with detectable levels in ≥50% of all samples and were investigated for differential expression against AF phenotypes by linear modeling (controlled for false discovery rate <0.05). Genes associated with AF susceptibility were determined by evaluating differential expression between AF/SR and NoAF groups. Genes associated with AF activity (persistence) demonstrated differential expression between AF/AF and AF/SR groups. Genes showing differential expression between NoAF and AF/AF were also investigated. Differentially expressed genes in this third comparison were associated with both AF susceptibility and persistent AF activity. The number and direction of differentially expressed gene probes for each contrast are summarized in Table 2, with the largest set of differentially regulated gene probes (2345) found for persistent AF activity comparison. The relative numbers of differentially expressed gene probes reflect both the transcriptional differences between phenotypes and the differences in sample sizes, and thus power. The top differentially expressed genes by AF phenotypes are shown in Figure 2.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>AF Phenotype</th>
<th>NoAF, n=32 (13%)</th>
<th>AF/SR, n=78 (33%)</th>
<th>AF/AF, n=129 (54%)</th>
<th>Total, n=239 (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age,* median (IQR), y</td>
<td>66.0 (57.3–72.5)</td>
<td>59.5 (53.3–65.0)</td>
<td>64.0 (55.0–70.0)</td>
<td>62.0 (55.0–69.0)</td>
</tr>
<tr>
<td>Female, n (%)*</td>
<td>12 (38)</td>
<td>21 (27)</td>
<td>26 (20)</td>
<td>59 (25)</td>
</tr>
<tr>
<td>History of CAD, n (%)*</td>
<td>17 (53)</td>
<td>57 (73)</td>
<td>87 (67)</td>
<td>161 (67)</td>
</tr>
<tr>
<td>History of MVD, n (%)*</td>
<td>21 (66)</td>
<td>44 (56)</td>
<td>57 (44)</td>
<td>122 (51)</td>
</tr>
<tr>
<td>LVEF, median (IQR), %</td>
<td>55.0 (48.8–60.0)</td>
<td>55.0 (55.0–60.0)</td>
<td>55.0 (50.0–55.0)</td>
<td>55.0 (50.0–60.0)</td>
</tr>
<tr>
<td>Left atrial size, median (IQR), cm</td>
<td>4.5 (4.1–5.1)</td>
<td>4.7 (4.2–5.2)</td>
<td>5.0 (4.3–5.8)</td>
<td>4.8 (4.2–5.3)</td>
</tr>
<tr>
<td>Duration of AF before study, median (IQR), mo</td>
<td>0</td>
<td>36.0 (8.0–75.0)</td>
<td>48.0 (12.0–96.0)</td>
<td>48.0 (12.0–96.0)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>3 (10)</td>
<td>10 (13)</td>
<td>14 (11)</td>
<td>27 (11)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>17 (53)</td>
<td>31 (40)</td>
<td>67 (52)</td>
<td>115 (48)</td>
</tr>
<tr>
<td>Heart failure, n (%)</td>
<td>4 (13)</td>
<td>14 (18)</td>
<td>37 (30)</td>
<td>55 (23)</td>
</tr>
<tr>
<td>Obesity, n (%)</td>
<td>22 (69)</td>
<td>51 (65)</td>
<td>97 (75)</td>
<td>170 (71)</td>
</tr>
<tr>
<td>Smoking history, n (%)</td>
<td>13 (41)</td>
<td>31 (40)</td>
<td>70 (54)</td>
<td>114 (48)</td>
</tr>
</tbody>
</table>

AF indicates atrial fibrillation; CAD, coronary artery disease; IQR, interquartile range; LVEF, left ventricular ejection fraction; MVD, mitral valve disease; NoAF, no history of AF; and SR, sinus rhythm.
*These variables were explicitly corrected for within the linear model for gene expression. Other covariates were balanced or implicitly corrected for by the factor analysis methodology.

Table 2. Summary of Differentially Expressed Probes

<table>
<thead>
<tr>
<th></th>
<th>AF Susceptibility: AF/SR vs NoAF</th>
<th>AF Activity and Susceptibility: AF/AF vs NoAF</th>
<th>AF Activity: AF/AF vs AF/SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differentially expressed probes</td>
<td>190</td>
<td>1011</td>
<td>2345</td>
</tr>
<tr>
<td>Increased expression (in first phenotype)</td>
<td>72</td>
<td>433</td>
<td>1043</td>
</tr>
<tr>
<td>Decreased expression (in first phenotype)</td>
<td>118</td>
<td>578</td>
<td>1302</td>
</tr>
</tbody>
</table>

AF indicates atrial fibrillation; NoAF, no history of AF; and SR, sinus rhythm.
The most significant differentially expressed mRNAs with their $P$ values and fold change effects are listed in Table 3 and a complete listing for all mRNAs meeting a false discovery rate cutoff of 0.05 is given in Table I in the Data Supplement. Fold changes, as listed in Tables 3 and 4 and Table I in the Data Supplement, are expression ratios comparing the first AF phenotype in the given comparison with the second.

To further characterize the transcriptional alterations associated with AF susceptibility and AF activity, gene set enrichment analysis was performed using romer, a competitive gene set test that estimates $P$ values by rotation tests. Specifically, for each pair of AF phenotype groups, gene sets were tested for enrichment in either up- or downregulated transcripts. Three gene set collections obtained from the Molecular Signatures Database...
<table>
<thead>
<tr>
<th>Illumina ID</th>
<th>Gene Name</th>
<th>P Value</th>
<th>FDR &lt;0.05 Adjusted P Value</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>ILMN_1772612</td>
<td>ANGPTL2</td>
<td>2.36E−23</td>
<td>2.78E−19</td>
<td>4.3</td>
</tr>
<tr>
<td>ILMN_1666411</td>
<td>FHL2</td>
<td>5.49E−23</td>
<td>3.24E−19</td>
<td>4.4</td>
</tr>
<tr>
<td>ILMN_2355831</td>
<td>FHL2</td>
<td>5.62E−22</td>
<td>2.21E−18</td>
<td>4.1</td>
</tr>
<tr>
<td>ILMN_1666385</td>
<td>CALM3</td>
<td>3.43E−21</td>
<td>1.01E−17</td>
<td>3.8</td>
</tr>
<tr>
<td>ILMN_1733998</td>
<td>DHR59</td>
<td>9.11E−21</td>
<td>2.15E−17</td>
<td>3.8</td>
</tr>
<tr>
<td>ILMN_1678643</td>
<td>FHL2</td>
<td>2.78E−18</td>
<td>5.47E−15</td>
<td>3.5</td>
</tr>
<tr>
<td>ILMN_1729980</td>
<td>RNF216</td>
<td>1.77E−16</td>
<td>2.98E−13</td>
<td>3.1</td>
</tr>
<tr>
<td>ILMN_2367239</td>
<td>RCAN1</td>
<td>3.56E−16</td>
<td>5.25E−13</td>
<td>3.2</td>
</tr>
<tr>
<td>ILMN_1783805</td>
<td>PNMA3</td>
<td>5.99E−16</td>
<td>7.85E−13</td>
<td>3.1</td>
</tr>
<tr>
<td>ILMN_1696675</td>
<td>CES2</td>
<td>1.04E−15</td>
<td>1.23E−12</td>
<td>3.2</td>
</tr>
<tr>
<td>ILMN_1793549</td>
<td>PTPN4</td>
<td>5.27E−15</td>
<td>5.65E−12</td>
<td>2.9</td>
</tr>
<tr>
<td>ILMN_2384181</td>
<td>DHR59</td>
<td>4.10E−14</td>
<td>4.03E−11</td>
<td>2.9</td>
</tr>
<tr>
<td>ILMN_2305544</td>
<td>DBI</td>
<td>8.49E−14</td>
<td>7.71E−11</td>
<td>3.0</td>
</tr>
<tr>
<td>ILMN_2301722</td>
<td>PDE8B</td>
<td>1.04E−13</td>
<td>8.75E−11</td>
<td>3.0</td>
</tr>
<tr>
<td>ILMN_2404049</td>
<td>RBM38</td>
<td>1.56E−13</td>
<td>1.23E−10</td>
<td>0.40</td>
</tr>
</tbody>
</table>

AF indicates atrial fibrillation; FDR, false discovery rate; and SR, sinus rhythm.
of the Broad Institute were investigated: A transcription factor collection (C3_TFT), where shared conserved cis-regulatory motifs (−2 kilobase pairs to +2 kilobase pairs from transcription start site) define gene sets, a miRNA collection (C3_MIR), where a shared 3′untranslated region motif defines each gene set, and a molecular function collection (C5_MF), where shared motif-based gene sets from the transcription factor and miRNA collections demonstrated numerous alterations associated with AF susceptibility, many of the identified gene sets were also associated with comparisons of NoAF and AF/AF samples (Table II in the Data Supplement).

Gene Set Enrichment Analysis of Persistent AF Activity

Motif-based gene sets from the transcription factor and miRNA collections associated with persistent AF activity were distinct from those associated with AF susceptibility. Transcription factor gene sets showed decreased expression of targets of transcription factors related to development such as HSF2, myogenic regulatory factor, and myocyte enhancer factor 2 in contrast with the findings in AF susceptibility (Tables 5 and 6).

In a large collection of human left atrial tissues, we evaluated the transcriptional changes associated with AF susceptibility by comparing samples from patients in sinus rhythm who differed by history of previous AF. We also examined transcriptional changes associated with ongoing AF by comparing changes in samples from patients in AF rhythm with those from patients with a history of AF in sinus rhythm. Although the latter comparison cannot distinguish changes that might predispose to persistent AF in patients with AF from those caused by AF, many of the changes corroborate prior electrophysiology studies of persistent AF. Gene set analysis of our transcriptome data implicates decreased activity of several

**Table 4. Notable Gene Sets and Groups of Gene Sets in AF Susceptibility (AF/SR vs NoAF)**

<table>
<thead>
<tr>
<th>Gene Sets</th>
<th>Gene Set Directionality in AF/SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>P53 targets (C3_TFT)</td>
<td>▼ Downregulated</td>
</tr>
<tr>
<td>CREB/ATF family targets (C3_TFT)</td>
<td>▼ Downregulated</td>
</tr>
<tr>
<td>SRF targets (C3_TFT)</td>
<td>▼ Downregulated</td>
</tr>
<tr>
<td>E2F1 targets (C3_TFT)</td>
<td>▼ Downregulated</td>
</tr>
<tr>
<td>ATF6 targets (C3_TFT)</td>
<td>▼ Downregulated</td>
</tr>
<tr>
<td>HSFl targets (C3_TFT)</td>
<td>▼ Downregulated</td>
</tr>
<tr>
<td>MEF2A targets (C3_TFT)</td>
<td>▲ Upregulated</td>
</tr>
<tr>
<td>MYOD targets (C3_TFT)</td>
<td>▲ Upregulated</td>
</tr>
<tr>
<td>mir499 targets (C3_MIR)</td>
<td>▲ Upregulated</td>
</tr>
<tr>
<td>Oxidoreductase activity (C5_MF)</td>
<td>▼ Downregulated</td>
</tr>
<tr>
<td>Cysteine type peptidase activity (C5_MF)</td>
<td>▼ Downregulated</td>
</tr>
<tr>
<td>Electron transport activity (C5_MF)</td>
<td>▲ Upregulated</td>
</tr>
</tbody>
</table>

AF indicates atrial fibrillation; NoAF, no history of AF; and SR, sinus rhythm.

**Table 5. Notable Gene Sets and Groups of Gene Sets in AF Activity (Persistence)**

<table>
<thead>
<tr>
<th>Gene Sets</th>
<th>Gene Set Directionality in AF/AF</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSFl2 targets (C3_TFT)</td>
<td>▼ Downregulated</td>
</tr>
<tr>
<td>MEF2A targets (C3_TFT)</td>
<td>▼ Downregulated</td>
</tr>
<tr>
<td>MYOD targets (C3_TFT)</td>
<td>▼ Downregulated</td>
</tr>
<tr>
<td>mir133 targets (C3_MIR)</td>
<td>▲ Upregulated</td>
</tr>
<tr>
<td>Ion Channel Activity, Gated</td>
<td>^ Downregulated</td>
</tr>
<tr>
<td>Channel Activity, Substrate</td>
<td>^ Downregulated</td>
</tr>
<tr>
<td>Specific Channel Activity, etc. (C5_MF)</td>
<td>^ Downregulated</td>
</tr>
</tbody>
</table>

**Gene Set Enrichment Analysis of Persistent AF Activity**

Motif-based gene sets from the transcription factor and miRNA collections associated with persistent AF activity were distinct from those associated with AF susceptibility. Transcription factor gene sets showed decreased expression of targets of transcription factors related to development such as HSF2, myogenic regulatory factor, and myocyte enhancer factor 2 in contrast with the findings in AF susceptibility (Tables 5 and 6). Concordant with prior functional electrophysiology studies, investigations of gene sets defined by molecular function revealed decreased expression of several gene sets related to ion channels in AF activity. Individual genes of significance within these sets related to various potassium channels and L-type calcium channel function are listed with their statistics in Table 6. The Ca2+ channel subunit genes CACNB2 and CACNA1C were downregulated with persistent AF activity. The inward rectifier K+ channel subunits KCNJ2 and KCNJ4 were upregulated, whereas KCNJ5 was downregulated with persistent AF activity. KCNN2, a member of the potassium small conductance calcium-activated channel family, was also downregulated with persistent AF activity. As with gene sets associated with AF susceptibility, many of the identified gene sets were also associated with comparisons of NoAF and AF/AF samples (Table II in the Data Supplement).

**Discussion**

In a large collection of human left atrial tissues, we evaluated the transcriptional changes associated with AF susceptibility by comparing samples from patients in sinus rhythm who differed by history of previous AF. We also examined transcriptional changes associated with ongoing AF by comparing changes in samples from patients in AF rhythm with those from patients with a history of AF in sinus rhythm. Although the latter comparison cannot distinguish changes that might predispose to persistent AF in patients with AF from those caused by AF, many of the changes corroborate prior electrophysiology studies of persistent AF. Gene set analysis of our transcriptome data implicates decreased activity of several
transcription factors known to be associated with the cellular stress response in AF susceptibility; however, there was little associated change in the expression of ion channels known to be involved in AF. In contrast, AF persistence was associated with numerous changes in ion channel expression at both the gene set and the individual probe level.

**Inflammation, Oxidation, and the Cellular Stress Response in AF Susceptibility**

Inflammation and oxidative stress are recognized to be involved in the pathophysiology of human AF in various contexts. For example, increased levels of C-reactive protein have been associated with postoperative AF, history of AF, lone AF, and AF risk. Similarly, postoperative AF has been correlated with myocardial protein oxidation, and decreased atrial glutathione content has been associated with AF history among patients in sinus rhythm at the time of cardiac surgery. Studies of postoperative AF have also demonstrated alterations in ratios of cardiac metabolites, which suggest that glycolytic inhibition, possibly because of oxidative stress, precedes the development of AF.

Animal models have further demonstrated mechanistic links between AF susceptibility and inflammatory and oxidative stress. Relative to controls, dogs treated with buthionine sulfoximine, an inhibitor of glutathione synthesis, had decreased atrial contractility, abbreviated effective refractory period, and reduced atrial myocyte L-type calcium current density (I_{ca,L}). Similar responses were also detected in a canine rapid atrial pacing model, where geranylgeranylacetone, an inducer of the CREB/ATF family, attenuated rapid-pacing–induced AF vulnerability and altered atrial contractility, abbreviated effective refractory period, and functional isoforms of ATF3 or CREM resulted in significant arrhythmogenesis. In separate murine models, overexpression of a nonphosphorylatable form of CREB, an inducer of the CREB/ATF family, or nonfunctional isoforms of ATF3 or CREM resulted in significant atrial dilation with development of AF or conduction abnormalities in the latter 3. Downregulation of several genetic loci involved with embryological development of the heart. In many patients, AF may only manifest in the setting of decreased/impaired responsiveness to prolonged cellular stress, decades after the genome-wide association studies identified AF loci exert their primary activity during cardiac and pulmonary vein development. Alternatively, these loci may be reactivated during the remodeling of the atria, as suggested by the increased expression of SRF.

**CREB/ATF Family**

Several animal studies have demonstrated that members of the CREB/ATF family are important in atrial dilatation, morphogenesis, and arrhythmogenesis. In separate murine models, overexpression of a nonphosphorylatable form of CREB, an inducer of the CREB/ATF family, or nonfunctional isoforms of ATF3 or CREM resulted in significant atrial dilation with development of AF or conduction abnormalities in the latter 3. Downregulation of the CREB/ATF family targets, as here detected in human atria, might be expected to lead to similar atrial structural and electric remodeling, thus predisposing to AF.

**Heat-Shock Factor 1**

The downregulation of HSF1 targets detected in this study is consistent with the AF susceptibility findings in experimental animal AF studies. Heat shock proteins protect cells...
from metabolic and thermal stresses. Brundel et al. demonstrated that the HSF1 inducer geranylgeranylacetone attenuated tachypacing-induced remodeling and susceptibility to pacing-induced AF in a canine model. They also showed that geranylgeranylacetone limited tachypacing-induced myolysis in HL-1 atrial myocytes by inducing heat shock proteins whose abundance was correlated with the duration of paroxysmal and persistent AF in human atrial tissue samples. In studies using pacing of HL-1 (atrial-derived) myocytes, geranylgeranylacetone was shown to induce synapse-associated protein 97 through HSF1 and thereby stabilize Kv1.5 channels. In the atria, Kv1.5 underlies I_kat1, a repolarizing current that we and others have found to be attenuated in persistent human AF.

ATF6
ATF6 is activated by the unfolded protein response and induces expression of cytoprotective ER stress proteins. In transgenic mice, preinduction of ATF6 expression has been demonstrated to improve echocardiographic and histological parameters after ischemia/reperfusion stress. Our finding of downregulation of ATF6 and HSF1 targets in human AF supports an impaired response to atrial myolysis and unfolded proteins, predisposing to damaged atrial proteostasis and remodeling. Other studies in human AF have shown that higher atrial expression of HSPB1 was related to shorter duration of AF episodes and less extensive myolysis, whereas longer duration AF may exhaust the HSP response. Our results are consistent with a likely benefit of upstream therapy for AF using inducers of heat shock proteins via HSF1 activation, as has been studied in animal models.

SRF
Targeted and timed recombinacion-mediated deletion models have demonstrated that SRF is an important transcription factor in both the embryonic and adult hearts. In embryonic hearts, SRF deletion leads to cardiac dilation, septation defects, and lethality. Notably, this phenotype is associated with significant reductions in other regulators of heart development such as NKX2.5 and GATA4, which have been associated with AF through genome-wide association studies. With respect to AF activity, we found increased expression of KCNJ4 and KCNJ2 in persistent AF provides some support for these channels as therapeutic targets in persistent AF, a condition in which there is likely limited use for blockers of other ion channel currents that are decreased in persistent AF (eg, I_n, I_Ca,L). Downregulation of KCNJ5, a component of G-protein–coupled inward rectifying potassium current (I_K,ATP), was also associated with persistent AF activity in concordance with prior studies of chronic human AF. This may represent a compensatory response to counteract parasympathetic-mediated shortening of the atrial effective refractory period in the setting of chronically high atrial rates.

Implied Activity of miRNAs in AF Activity
In recent years, the contribution of miRNA activity to AF has been demonstrated in models of PITX2c insufficiency and AF induced remodeling. As miRNAs can promote mRNA degradation by activating endonuclease cleavage and facilitating decapping, miRNA activity can be inferred from our transcriptional data. With respect to AF activity, we found increased expression of mir-133 targets associated with persistent AF in comparisons of AF/AF with other groups implying decreased mir-133 activity. Interestingly, mir-1 and mir-133 are derived from a common precursor transcript and mir-1 levels have been demonstrated to be reduced in AF with subsequent increases in inward-rectifier potassium currents. In canine models of AF which examined the impact of nicotine in promoting remodeling in the setting of rapid pacing induced AF, mir-133 downregulation was found to mediate the profibrotic response. We have also demonstrated that mir-133 has increased the expression in the left atria relative to the right atria, suggesting that persistent AF may alter the differentiation of the left atria through this miRNA.

This finding of decreased mir-133 activity associated with persistent AF activity also correlates with our transcription factor analysis, as myocyte enhancer factor 2A is a known inducer of mir-1 and mir-133 in cardiac and skeletal muscle and also shows decreased activity in the persistent AF activity comparison. Increased expression of myocyte enhancer factor 2A targets in AF/SR samples relative to both AF/AF and NoAF samples may represent a compensatory response...
Conclusions

AF susceptibility, independent of changes induced by AF rhythm itself, is associated with significant transcriptional remodeling related to the decreased expression of pathways associated with inflammation, oxidation, and generic cellular stress responses. Conversely, our data imply that stress-induced transcriptional responses may decrease AF susceptibility. Although our results are limited to the transcriptome, these stresses have been previously associated with susceptibility to AF by our group and others in targeted protein and functional studies. This suggests a mechanism by which AF manifests after exposure to inflammatory and oxidative stress with an insufficient transcriptional response and may help to explain why AF often does not manifest until stresses develop in older age, despite genetic predispositions. As the prevalence of comorbid disease states which could contribute to inflammatory and oxidative stress was similar between those groups involved in the AF susceptibility comparison, the difference in transcriptional response may be because of genetic differences.

In addition, significant alterations in ion channel transcription were limited to samples with persistent AF. This implies that changes in ion channel transcription occur later in the progression of AF, or as a consequence of AF, and the directionality of expression changes suggest several potential therapeutic targets. Our results further suggest that several miRNA species (miR-1, miR-133) are likely important mediators or modulators of the observed transcriptional changes. These results have identified pathways that can inform future studies using novel therapeutic or preventive strategies.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Supplemental Methods 1. Interpretation of top gene sets with overlapping member genes

In most curated collections of gene sets appropriate for use in gene set analysis, there is frequent and varying overlap between the gene sets of a collection such that, given any two gene sets in a collection, they may share all, few, or no genes in common. This creates a challenge in interpreting the results of gene set differential expression enrichment analyses as multiple gene sets containing the same differentially expressed genes may be identified as significant.

To address this difficulty, top gene sets were clustered on the basis of dissimilarity and then analyzed in groups when appropriate as follows: Let \( A \) and \( B \) be gene sets of the same collection (C3_TFT, C3_MIR, C5_MF) whose member genes may overlap. Then the dissimilarity between \( A \) and \( B \) can be calculated as:

\[
Dissimilarity(A, B) = \frac{\# \text{ of genes unique to } A \text{ or } B}{\# \text{ of genes in } A \text{ or } B}
\]

Therefore, when \( A \) and \( B \) are identical, their dissimilarity is 0 and the maximum dissimilarity is 1. Further the dissimilarity caused by one unique gene between sets is dependent on the overall size of the sets in question.

Using this notion of dissimilarity, or distance. It is then possible to cluster top gene sets showing enrichment for genes with either increased or decreased expression for a given AF phenotype comparison. Clustering was done using a complete linkage method as it creates cluster which are tightly clustered. On the basis of clusters of gene sets resulting from this process, gene sets or groups of gene sets were considered for biological significance in the AF phenotype comparison they were associated with.

Supplemental Table 1. Top Probes For Each AF Phenotype Comparison

Supplemental Table 1.xlsx file provided separately.

Supplemental Table 2. Top Gene Sets For Each AF Phenotype Comparison

Supplemental Table 2.xlsx file provided separately.