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Left Atrial Transcriptional Changes Associated with Atrial Fibrillation Susceptibility and Persistence

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Abstract

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 AF history, AF history in sinus rhythm at surgery, 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 (TF) collection, defined by shared conserved cis-regulatory motifs; a miRNA collection, defined by shared 3′UTR 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, HSF1, ATF6, SRF, and E2F1 TFs. Persistent AF activity was associated with decreased

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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, while remodeling of ion channel expression occurs later in the progression or as a consequence of AF.

Keywords

atrial fibrillation; transcription; transcriptional regulation; transcriptome

Introduction

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 electrical, 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.

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, 2] As the insults leading to AF are varied and AF is associated with AF-initiated pro-arrhythmic changes, the unifying mechanisms that precede chronic forms of AF remain uncertain. While recent genome-wide association studies have implicated loci near or in embryonic 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 ion channels and components of cellular signaling cascades.[3,4] Moreover, prior trancriptomic, proteomic, and metabalomic investigations of AF have been mostly limited to animal models, right atria, and chronic AF.[5–9] While these studies have correlated transcriptional 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 contributes.

Approximately 70–80% of patients with AF have structural heart disease.[1] 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 non-persistent 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 (LAA) tissues obtained from patients who underwent cardiac surgery at the Cleveland Clinic and who consented to have discarded tissue used for research. LAA tissues were also obtained from two donor hearts without AF that were unsuitable for transplantation. Donor families consented to use of the tissues for research. Tissue collection and investigations were performed in accordance with a Cleveland Clinic IRB approved protocol. Samples were stored at −80°C prior to RNA extraction.

Three AF phenotypes were determined by review of AF history and electrocardiograms taken just prior to 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 to NoAF samples, whereas AF/AF compared to AF/SR samples reflected persistent AF activity, and AF/AF samples reflected both AF susceptibility and persistent AF activity when compared to 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 cRNA and biotin-UTP labeled using the TotalPrep RNA Amplification Kit (Ambion, Austin, TX). cRNA was quantified using a Nanodrop spectrophotometer and cRNA size distribution was assessed on a 1% agarose gel. cRNA 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 at least 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 gender, age, CAD, and mitral valve disease (MVD).

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 un-modeled 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 (FDR) <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 (gender, age, CAD, and mitral valve disease). The residuals were aggregated towards the medians to generate gene level expression values. Finally expression values were normalized by dividing by the standard deviation 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 by utilizing 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 (100,000 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 employs a parametric re-sampling 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 Supplemental Methods 1.

Results

Gene-wise Differential Expression by AF phenotype

Of the 49,576 probes assayed, after filtering, 11,806 were expressed with detectable levels in at least 50% of all samples and were investigated for differential expression against AF

phenotypes by linear modeling (controlled for FDR <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 is summarized in Table 2, with the largest set of differentially regulated gene probes (2,345) 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.

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 FDR cutoff of 0.05 is given in Supplemental Table 1. Fold changes, as listed in Tables 3, 5 and Supplemental Table 1 are expression ratios comparing the first AF phenotype in the given comparison to 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 which estimates p-values by rotation tests. Specifically, for each pair of AF phenotype groups, gene sets were tested for enrichment in either up or down-regulated transcripts. Three gene set collections obtained from the Molecular Signatures Database 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′UTR motif defines each gene set, and a “molecular function” collection (C5_MF), where shared Gene Ontology molecular function ontology defines gene sets[8].

In all three collections, gene set membership is non-exclusive. As a result, gene sets with substantial overlap in members had similar p-values. This is especially true among the top ranking gene sets. Top gene sets were considered as clusters, as described in Supplemental Methods 1. Notable gene sets and clusters of gene sets associated with AF susceptibility and persistent AF activity are summarized in Table 4 and 5. A comprehensive listing of the top ranking gene-sets for each comparison is provided in Supplemental Table 2.

Gene-set enrichment analysis of AF susceptibility (Table 4)

Motif based gene sets from the transcription factor and miRNA collections demonstrated numerous alterations associated with AF susceptibility. Many of the transcription factors and miRNAs identified in this analysis have been implicated in cellular stress responses. Specifically, targets of transcription factors known to be part of the response to oxidant stress, such as CREB, showed decreased expression in AF susceptibility. Similarly, targets of HSF1, involved in the heat shock response, and ATF6, involved in the unfolded protein response, had decreased expression. In addition, targets of transcription factors known to be modulators of the cell cycle such as P53, E2F1 and SRF had decreased expression. Other

transcription factors identified in AF susceptibility included myogenic regulatory factor, MYOD and myocyte enhancer factor, MEF2. The targets of these transcription factors showed increased expression in AF susceptibility.

These findings were complemented by investigations of molecular functions associated with AF susceptibility which included down-regulation of genes associated with oxidoreductase activity and cysteine peptidase activity and up-regulation of lyase (eg., decarboxylase, aldolase, cyclase) and electron transport activity. Further, many of the above gene sets were enriched for genes showing differential expression between AF/AF and noAF samples (Supplemental Table 2).

Gene-set enrichment analysis of persistent AF activity (Table 5, 6)

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 heat-shock factor 2 (HSF2), MYOD, and MEF2 in contrast with the findings in AF susceptibility.

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 Ca^{2+} channel subunit genes CACNB2 and CACNA1C were down-regulated with persistent AF activity. The inward rectifier K^{+} channel subunits KCNJ2 and KCNJ4 were upregulated, while KNCJ5 was down-regulated with persistent AF activity. KCNN2, a member of the potassium small conductance calcium-activated channel family, was also down-regulated 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 (Supplemental Table 2).

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 to 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 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[12]. For example, increased levels of C-reactive protein have been associated with post-operative AF[13], history of AF, lone AF[14], and AF risk[15]. Similarly, postoperative AF has been correlated with myocardial protein oxidation,[16] and decreased atrial glutathione content has been associated with AF history among patients in sinus rhythm at the time of cardiac surgery[17]. Studies of post-operative AF have also demonstrated alterations in ratios of cardiac metabolites which suggest that glycolytic inhibition, possibly due to oxidative stress precedes the development of AF.[9]

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 ($I_{Ca,L}$) [17]. Similar responses were also detected in a canine rapid atrial pacing model, where geranylgeranylacetone (GGA), an inducer of HSF1 (heat shock transcription factor 1), attenuated rapid-pacing induced AF vulnerability and decreases in atrial myocyte action potential duration and $I_{Ca,L}$ density [18]. In a proteomic and metabolomic study of a ventricular pacing canine model of heart failure and AF, left atrial samples demonstrated decreased expression of antioxidants, alterations in glucose metabolism and accompanying fragmentation in atrial structural proteins.[8]

Consistent with this literature, relative to those without AF, patients with history of AF were characterized by decreased expression of targets of several transcription factors known to be activated in the cellular stress response, including the CREB/ATF family, HSF1, ATF6, SRF, E2F1 and SP1 transcription factors. In addition, investigations of GO molecular functions demonstrated decreased expression of gene sets associated with oxidoreductase activity and cysteine peptidase activity in samples from patients with AF susceptibility. NoAF samples were obtained from patients with similar concomitant cardiovascular risk factors as the AF/SR patient samples including diabetes, hypertension, heart failure, obesity, and smoking history (Table 1). Thus, both likely had similar background exposure to oxidative and inflammatory stressors. Further, differences in MVD and CAD were explicitly adjusted for in the differential expression model.

Together, these results suggest that rather than the modeled risk factors or AF rhythm itself, *AF susceptibility is associated with decreases in several transcriptional responses to cellular stresses*. This observation may help to explain why AF is primarily a disease of older ages, despite the significant association with AF through genome wide association studies (GWAS) of several genetic loci involved with embryologic 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 GWAS 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. Below, we summarize the existing literature regarding the

cardiac significance of the specific cell stress response transcription factors discovered in our work.

CREB/ATF family

Several animal studies have demonstrated that members of the CREB/ATF family are important in atrial dilation, morphogenesis, and arrhythmogenesis. In separate murine models, overexpression of a non-phosphorylatable form of CREB[19], JDP2[20] (an inhibitor of the CREB/ATF family), or non-functional isoforms of ATF3[21] or CREM[22] resulted in significant atrial dilation with development of AF or conduction abnormalities in the latter three. Down- regulation of the CREB/ATF family targets, as here detected in human atria, might be expected to lead to similar atrial structural and electrical remodeling, thus predisposing to AF.

HSF1

The down regulation 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 and colleagues demonstrated that the HSF1 inducer GGA attenuated tachypacing induced remodeling and susceptibility to pacing-induced AF in a canine model[18]. They also showed that GGA 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[23]. In studies using pacing of HL-1 (atrial-derived) myocytes, GGA was shown to induce synapse associated protein 97 through HSF1 and thereby stabilize Kv1.5 channels.[24] In the atria, Kv1.5 underlies I_{Kur}, a repolarizing current that we and others have found to be attenuated in persistent human AF.[25]

ATF6

ATF6 is activated by the unfolded protein response and induces expression of cytoprotective ER stress proteins. In transgenic mice, pre-induction of ATF6 expression has been demonstrated to improve echocardiographic and histological parameters after ischemia/reperfusion stress.[26]

Our finding of down-regulation of ATF6 and HSF1 targets in human AF supports an impaired response to atrial myolysis and unfolded proteins, predisposing to deranged 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.[23] 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.[27]

SRF

Targeted and timed recombination-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. [28] 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.[28] Temporally controlled disruption of SRF expression in the adult heart also led to contractile dysfunction, dilation and heart failure.[29]

Electrical remodeling in persistent AF is associated with differential expression of ion channels

While we did not detect differences in ion channel expression associated with AF susceptibility in comparisons between NoAF and AF/SR samples, such changes were prominent in samples from patients with persistent AF. In agreement with previously reported functional and transcriptional studies, relative to other samples, Table 6 shows that those in AF (AF/AF) demonstrated decreased expression of KCNN2 (SK2 channel), KCNJ5 (GIRK4KATP/G-protein coupled K^+ channel subunit) and L-type calcium channel subunits (CACNA1C and CACNB2) and increased expression of KCNJ2 (Kir2.1).[4, 5,30,31, 32] We previously documented downregulation of L-type calcium currents in human atrial myocytes. [33] We also found increased expression of KCNJ4/Kir2.3 in AF/AF samples, not previously reported in right atrial transcriptional studies of AF, but consistent with our functional studies.[5,25]

Given that both KCNJ2 and KCNJ4 contribute to the inward rectifying potassium current (I_{K1}) that is responsible for atrial myocyte resting potential, increased expression would be expected to shorten atrial refractory period. Gain-of-function mutations of KCNJ2 have been associated with familial forms of AF, and it is feasible that increased I_{K1} (associated with KCNJ2/4 up regulation) contributes to the stabilization/perpetuation of AF.[31] KCNJ4 has been identified as a potential target for anti-arrhythmic drugs, as it is expressed at nearly 10-fold greater levels in the atria relative to the ventricles of animal models.[34] 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 utility for blockers of other ion channel currents that are decreased in persistent AF (eg., I_{Na}, $I_{Ca,L}$). Down-regulation of KCNJ5, a component of G-protein coupled inward rectifying potassium current ($I_{K,ACh}$), 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.[32]

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[35] and AF induced remodeling[36, 37]. As miRNAs can promote mRNA degradation by activating endonuclease cleavage and facilitating de-capping, 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[37]. In canine models of AF which examined the impact of nicotine in promoting remodeling in the setting of rapid pacing induced AF, mir-133 down regulation

was found to mediate the pro-fibrotic response.[38] We have also demonstrated that mir-133 has increased expression in the left atria relative to the right atria, suggesting persistent AF may alter the differentiation of the left atria through this miRNA.[39]

This finding of decreased mir-133 activity associated with persistent AF activity also correlates with our transcription factor analysis, as MEF2A 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[40]. Increased expression of MEF2A targets in AF/SR samples relative to both AF/AF and noAF samples may represent a compensatory response that prevents AF susceptibility from leading to persistent AF (Tables 4, 5).

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 co-morbid 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 due to 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 modifiers 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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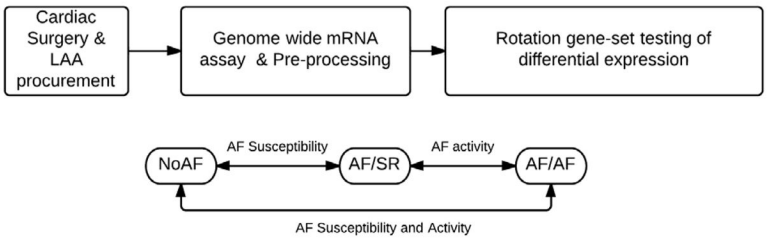
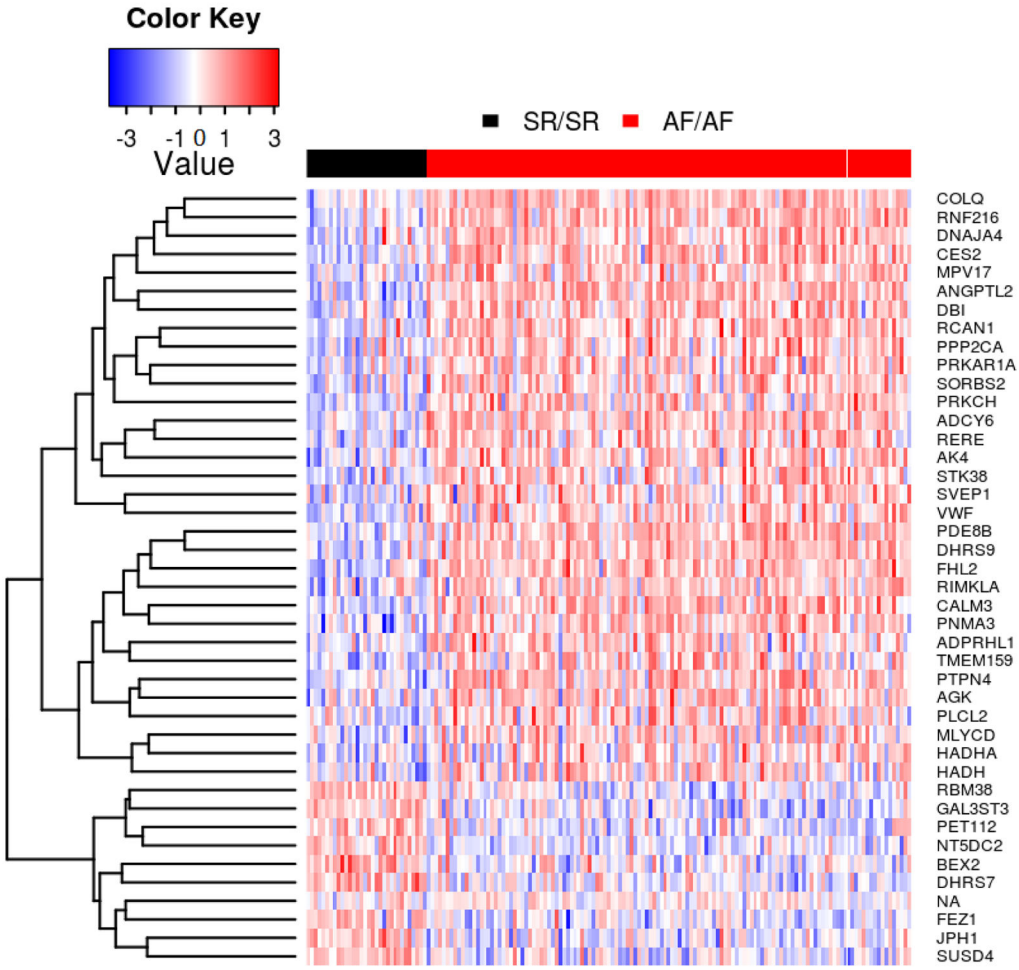


Figure 1.
Study design

Figure 2A.



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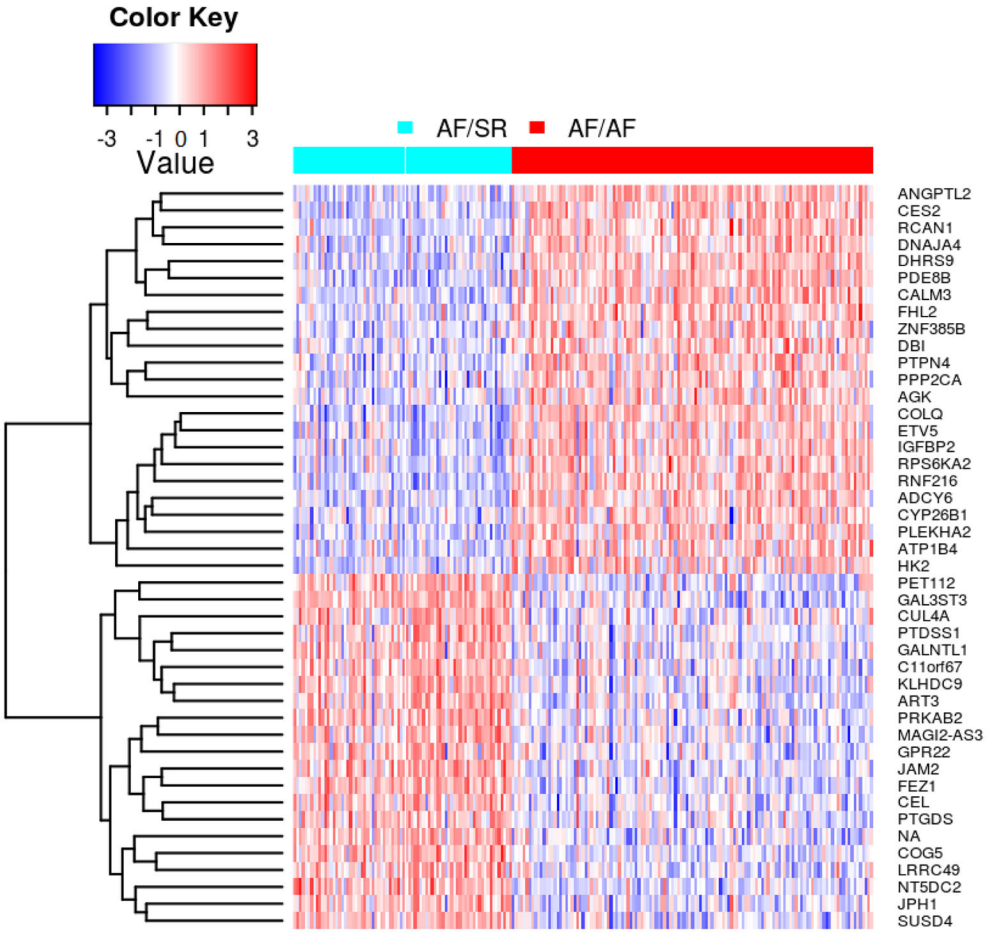

Figure 2C

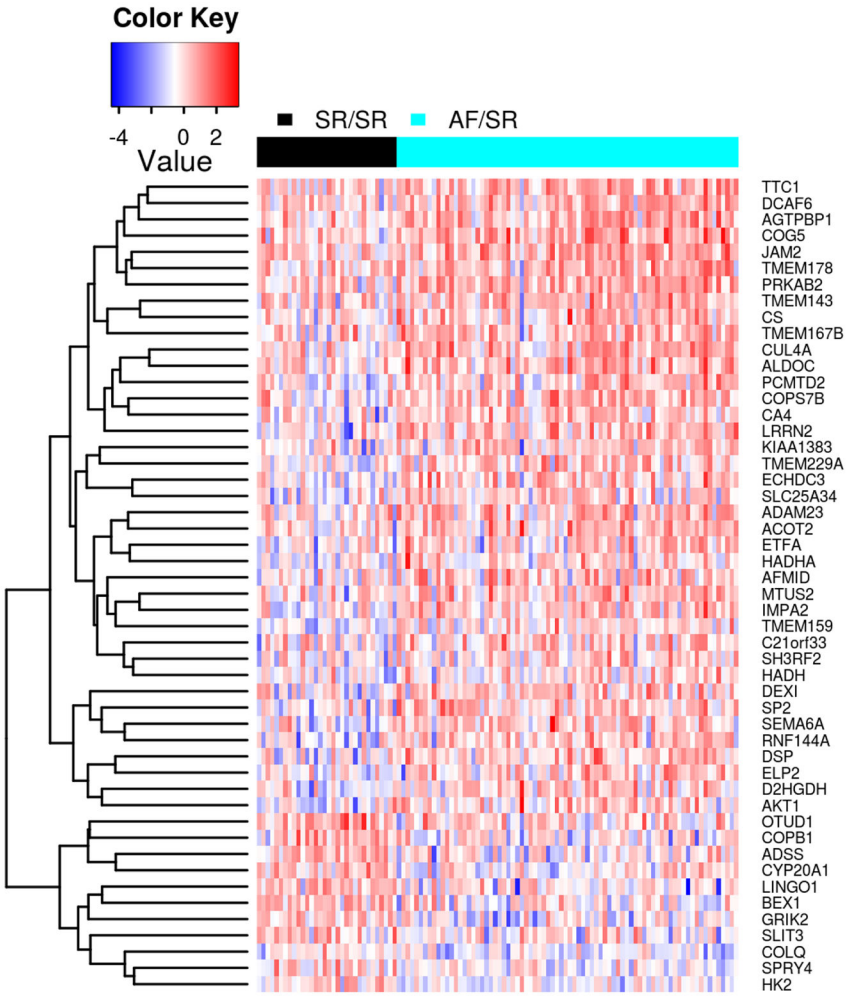

Figure 2D

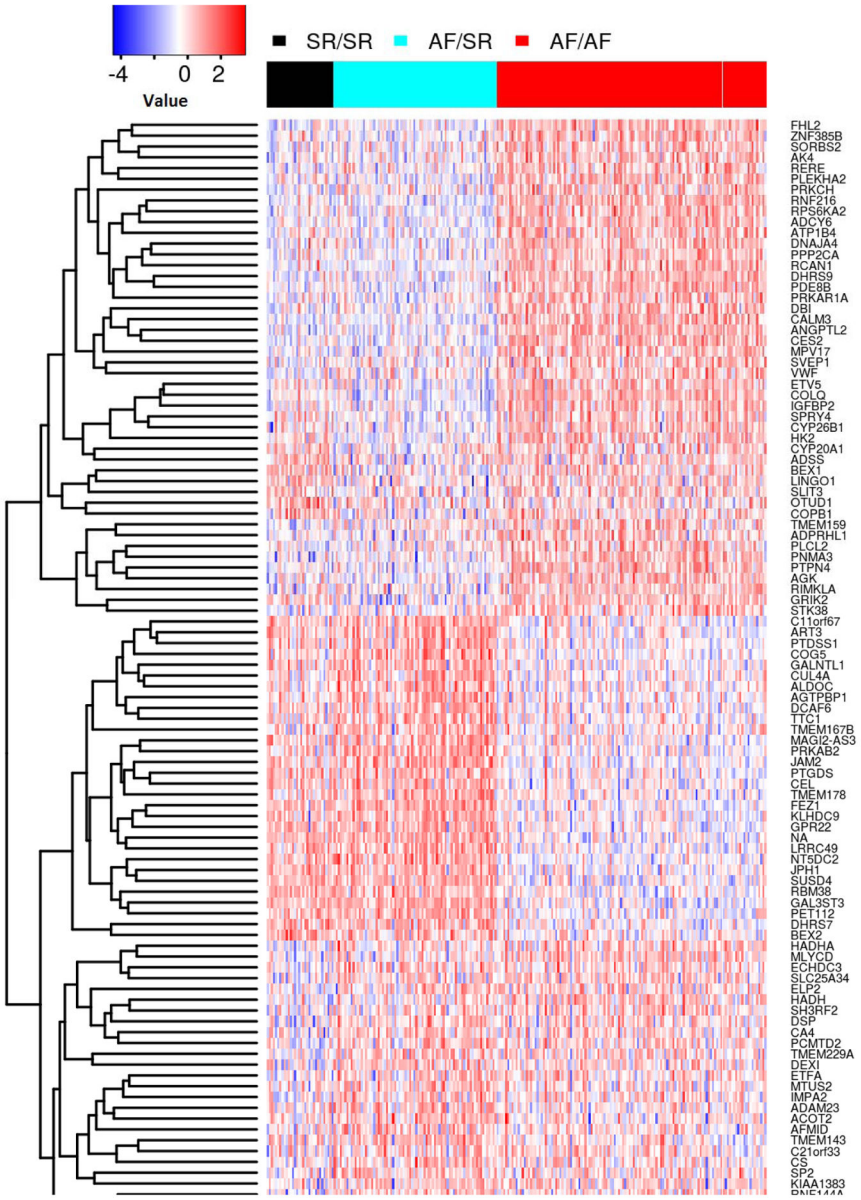

Figure 2.

Top 50 differentially expressed genes by AF history and rhythm at surgery: A. SR/SR vs AF/AF. B. AF/SR vs AF/AF. C. SR/SR vs AF/SR. D. SR/SR vs AF/SR vs AF/AF. Columns represent samples and rows represent genes.

Table 1

Patient characteristics

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

CAD = coronary artery disease. MVD = mitral valve disease. LVEF=left ventricular ejection fraction. IQR = interquartile range. AF = atrial fibrillation. SR = sinus rhythm.

*These variables were explicitly corrected for within the linear model for gene expression. Other co-variates were balanced or implicitly corrected for by the factor analysis methodology.

Table 2

Summary of differentially expressed probes

	AF susceptibility: AF/SR vs. NoAF	AF activity & susceptibility: AF/AF vs. NoAF	AF activity: AF/AF vs. AF/SR
Differentially Expressed Probes	190	1011	2345
Increased Expression (in 1st phenotype)	72	433	1043
Decreased Expression (in 1st phenotype)	118	578	1302

Table 3

Top differentially expressed probes in AF phenotype comparisons

AF/AF vs. NoAF (AF Susceptibility & Activity)				
Illumina ID	**Gene Name**	**p-value**	**FDR<0.05 adjusted p-value**	**Fold Change**
ILMN_1772612	ANGPTL2	2.36E-23	2.78E-19	4.3
ILMN_1668411	FHL2	5.49E-23	3.24E-19	4.4
ILMN_2355831	FHL2	5.62E-22	2.21E-18	4.1
ILMN_1666385	CALM3	3.43E-21	1.01E-17	3.8
ILMN_1733998	DHRS9	9.11E-21	2.15E-17	3.8
ILMN_1678643	FHL2	2.78E-18	5.47E-15	3.5
ILMN_1729980	RNF216	1.77E-16	2.98E-13	3.1
ILMN_2367239	RCAN1	3.56E-16	5.25E-13	3.2
ILMN_1783805	PNMA3	5.99E-16	7.85E-13	3.1
ILMN_1696675	CES2	1.04E-15	1.23E-12	3.2
ILMN_1793549	PTPN4	5.27E-15	5.65E-12	2.9
ILMN_2384181	DHRS9	4.10E-14	4.03E-11	2.9
ILMN_2305544	DBI	8.49E-14	7.71E-11	3.0
ILMN_2301722	PDE8B	1.04E-13	8.75E-11	3.0
ILMN_2404049	RBM38	1.56E-13	1.23E-10	0.40

AF/SR vs. NoAF (AF Susceptibility)				
Illumina ID	**Gene Name**	**p-value**	**FDR<0.05 adjusted p-value**	**Fold Change**
ILMN_1767848	PCMTD2	1.09E-10	1.28E-06	2.2
ILMN_1786021	PRKAB2	2.81E-09	1.66E-05	2.3
ILMN_1695792	CUL4A	4.22E-09	1.66E-05	2.0
ILMN_1696657	LRRN2	1.31E-08	3.17E-05	2.3
ILMN_2094061	IMPA2	1.39E-08	3.17E-05	2.2
ILMN_1718924	ETFA	1.61E-08	3.17E-05	1.9
ILMN_1721535	COG5	3.91E-08	6.60E-05	2.1
ILMN_1659749	DSP	7.33E-08	0.000108	2.0
ILMN_2072178	ECHDC3	9.46E-08	0.000124	2.1
ILMN_1777660	RNF144A	4.81E-07	0.000568	2.1
ILMN_1661376	GRIK2	6.80E-07	0.00073	0.50
ILMN_1655876	TMEM159	1.11E-06	0.001021	2.0
ILMN_2234697	BEX1	1.14E-06	0.001021	0.50
ILMN_1666482	SP2	1.28E-06	0.001021	2.0
ILMN_1695157	CA4	1.30E-06	0.001021	1.9

AF/AF vs. AF/SR (AF Activity)				
Illumina ID	**Gene Name**	**p-value**	**FDR<0.05 adjusted p-value**	**Fold Change**
ILMN_1729980	RNF216	1.63E-41	1.93E-37	4.2

AF/AF vs. AF/SR (AF Activity)				
Illumina ID	**Gene Name**	**p-value**	**FDR<0.05 adjusted p-value**	**Fold Change**
ILMN_2329114	COLQ	3.22E-40	1.90E-36	4.2
ILMN_1695991	COLQ	5.38E-34	2.12E-30	3.6
ILMN_1779071	FEZ1	1.06E-33	3.11E-30	0.30
ILMN_1772612	ANGPTL2	9.48E-32	2.24E-28	3.5
ILMN_2301722	PDE8B	1.20E-31	2.37E-28	3.7
ILMN_1697081	GAL3ST3	1.53E-31	2.58E-28	0.30
ILMN_2367239	RCAN1	7.34E-31	1.08E-27	3.3
ILMN_1666385	CALM3	4.16E-30	5.46E-27	3.2
ILMN_1708743	NT5DC2	2.77E-29	3.27E-26	0.31
ILMN_1696675	CES2	4.58E-29	4.91E-26	3.3
ILMN_1733998	DHRS9	7.28E-29	7.17E-26	3.1
ILMN_2311674	ADCY6	6.47E-28	5.88E-25	3.1
ILMN_1672350	JAM2	1.13E-27	9.52E-25	0.33
ILMN_1725193	IGFBP2	1.26E-27	9.92E-25	2.8

Table 4

Notable gene sets and groups of gene sets in AF susceptibility (AF/SR vs. NoAF)

Gene sets	Gene set directionality in AF/SR
P53 targets (C3_TFT)	▼ Down-regulated
CREB/ATF family targets (C3_TFT)	▼ Down-regulated
SRF targets (C3_TFT)	▼ Down-regulated
E2F1 targets (C3_TFT)	▼ Down-regulated
ATF6 targets (C3_TFT)	▼ Down-regulated
HSF1 targets (C3_TFT)	▼ Down-regulated
MEF2A targets (C3_TFT)	▲ Up-regulated
MYOD targets (C3_TFT)	▲ Up-regulated
mir499 targets (C3_MIR)	▲ Up-regulated
Oxidoreductase activity (C5_MF)	▼ Down-regulated
Cysteine type peptidase activity (C5_MF)	▼ Down-regulated
Electron transport activity (C5_MF)	▲ Up-regulated

Table 5

Notable gene sets and groups of gene sets in AF activity (persistence)

Gene sets	Gene set directionality in AF/AF
HSF2 targets (C3_TFT)	▼ Down-regulated
MEF2 targets (C3_TFT)	▼ Down-regulated
MYOD targets (C3_TFT)	▼ Down-regulated
mir133 targets (C3_MIR)	▲ Up-regulated
Ion Channel Activity, Gated Channel Activity, Substrate Specific Channel Activity, etc. (C5_MF)	▼ Down-regulated

Table 6

Individual genes of importance in AF activity (persistence, AF/AF vs. AF/SR) within ion channel related gene sets*

	AF/SR vs. NoAF		AF/AF vs. AF/SR		AF/AF vs. NoAF	
Gene symbol	**Fold change**	**P-value and (FDR<0.05 adjusted)**	**Fold change**	**P-value and (FDR<0.05 adjusted)**	**Fold change**	**P-value and (FDR<0.05 adjusted)**
KCNN2	1.08	0.58 (0.84)	0.60	1.7E-5 (2.9 E-4)	0.65	7.0E-4(3.5E-3)
KCNJ5	0.97	0.83 (0.95)	0.71	1.3E-6(3.1E-5)	0.69	0.01 (0.10)
KCNJ2	1.15	0.35 (0.71)	1.64	1.1E-3 (9.2E-3)	1.89	1.2E-5 (6.0E-4)
KCNJ4	1.35	0.03 (0.26)	1.35		1.82	5.3E-6 (3.3E-4)
CACNB2	1.49	0.001 (0.06)	0.58	1.3E-10 (8.5 E-9)	0.86	0.21(0.52)
CACNA1C	1.30	0.06 (0.36)	0.64	1.7E-6(4.0E-5)	0.83	0.16(0.45)

* For genes mapped to by multiple probes, statistics given for probe with highest average expression