Changes in the Atrial Transcriptome and Atrial Fibrillation
Susceptibility, Persistence, Causes, and Consequences

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Atrial fibrillation (AF) is a common clinical problem, presenting the dual challenges of increasing prevalence and therapeutic complexity. An improved understanding of the underlying pathophysiology is central to improving management options for the arrhythmia. As AF persists over time, complications increase and treatment becomes more difficult, and it has been suggested that aggressive early intervention might be valuable in improving outcomes. Different AF forms have distinct properties and mechanisms. More information is needed about the determinants of the various forms of AF, to improve risk prediction and develop new therapeutic options.

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Experimental and clinical studies indicate that different types of AF are characterized by distinct gene expression patterns. Right atrial samples from patients with valvular heart disease and long-standing clinical AF showed differences in 169 genes relative to patients with valvular heart disease in sinus rhythm, with particularly marked alterations in genes related to gene/protein synthesis, contraction/cytoskeleton, immune/inflammatory response, metabolism, fibrosis, and ion channels. One limitation to interpreting observations in patients with long-standing AF is that gene expression differences that predispose to AF cannot be separated from those that result from AF. In a study of ion-channel genomics in AF, a patient sample with sinus rhythm restoration was used to analyze the reversibility of changes, with the presumption that alterations that normalized with sinus rhythm were because of, rather than causes of, AF: all the changes examined proved to be reversible.

Study of Transcriptome Differences Associated With AF Susceptibility and Persistence

In this issue of Circ Arrhythm Electrophysiol, Deshmukh et al present the results of an interesting and elegant analysis of the atrial transcriptome (the global pattern of mRNA expression) in clinical AF. RNA extracted from left atrial samples of 239 patients was subjected to detailed analysis with the use of pangenomic mRNA microarrays: 32 patients had no AF history (no-AF group), 78 had a history of AF but were in sinus rhythm at the time of cardiac surgery (AF/SR group), and 129 had a history of AF and were in AF during surgery (AF/AF group). AF/SR patients were assumed to reflect AF susceptibility, whereas AF/AF patient properties were attributed to AF persistence superimposed on AF susceptibility. The groups were reasonably matched for sex, history of heart disease, left ventricular function, and smoking history. The AF/SR patients were younger than the other groups (mean age, 59.5 versus 64 and 66 years for AF/AF and no-AF, respectively). The influences of age, sex, and history of heart disease were corrected mathematically in gene expression analysis. AF history was long-standing in both AF groups, averaging 36 months in AF/SR and 48 months in AF/AF.

Of the 11,806 probe sets expressed at detectable levels in >50% of samples, 190 were differential between AF/SR and no-AF (reflecting AF susceptibility) and 2345 were differential between AF/AF and AF/SR (reflecting AF persistence); curiously, the latter figure is larger than the number of differential genes between AF/AF and no-AF (1011). Targets of transcription factors activated by oxidant stress were downregulated in AF/SR, as were targets of heat-shock factor 1 and activating transcription factor 6 (components of the heat-shock response and endoplasmic reticulum stress response, respectively). Molecular function categories downregulated in AF/SR versus no-AF included oxidoreductase and cysteine peptidase activity; lyase and electron transport activity genes were upregulated. A variety of ion-channel gene changes known to be caused by AF and to promote AF persistence were noted in AF/AF versus AF/SR, including upregulation of KCNJ2 and KCNJ4 (encoding Kir2.1 and Kir2.3 subunits, respectively, which contribute to I_{K1}) and downregulation of CACNA1C (encoding the I_{CaL}α-subunit) and CACNB2 (an I_{CaL}β-subunit).

This study presents several novel and important elements. First, it includes by far the largest number of AF patient samples of any study to date examining the AF-related transcriptome. Because interpatient variability is potentially significant by virtue of differences in cardiac disease, drug therapy, age, etc, large sample sizes provide the best chance of ensuring reliable and generalizable results. Second, the analytic approaches applied were sophisticated. Third, the use of left atrial tissue is important because the left atrium is considered to be particularly important in AF maintenance and most prior human studies have used right atrial tissues. Fourth, the specific populations studied are interesting. The inclusion of a group of patients with a history of AF but not in AF at the time of surgery adds an important novel element lacking in most
prior analyses of gene expression changes in AF, because it presumably removes the influence of AF-induced remodeling.

The mechanistic basis of the different clinical forms of AF is poorly understood. The duration and frequency of AF episodes vary widely among individuals, with significant implications for management and prognosis. AF is generally considered paroxysmal if it self-terminates within 7 days, persistent if it lasts >7 days and long-standing persistent if it endures for much longer periods, typically for a year. Attrial tissue samples are much easier to obtain from patients with persistent AF than those with the paroxysmal form, because the types of serious heart diseases that require surgical intervention allowing for atrial tissue sampling tend to cause persistent AF. Thus, investigations providing information about the molecular characteristics of atria from patients with paroxysmal AF, or at least a history of AF but no AF-induced remodeling, are much needed.

The present study provides valuable new information by sampling patients with a history of AF and sinus rhythm during cardiac surgery, and contrasting their results with those in patients without an AF history and in patients with a history of AF who maintain AF through surgery. The AF/AF group clearly had long-standing persistent AF, with the expected ion-channel subunit remodeling changes. It is tempting to equate the AF/AF group with paroxysmal AF, but because the durations of AF episodes are not documented for AF/SR patients (and were presumably not known), this equation would be incorrect. For example, patients with AF that had lasted for weeks or months and then been cardioverted would be in the AF/AF group, despite having clearly manifested persistent AF. Nevertheless, the AF/SR population was clearly distinct from the AF/AF population: when both were compared with no-AF, AF/SR patients had less than one fifth the number of differentially expressed genes versus the AF/AF group. In addition, AF/SR patients had none of the ion-channel gene expression changes expected with AF-induced remodeling.

It seems reasonable to suggest, as do the authors, that the AF/SR patients manifested AF susceptibility, because all of them had a history of documented AF. However, it would probably be wrong to suggest that these individuals are representative of the broad population of AF-susceptible individuals. Paroxysmal AF presents in different clinical forms, pointing to discrete underlying mechanisms. Patients with an apparent preponderance of automatic mechanisms (frequent short episodes, with persistence documented rarely if at all) often lack significant structural heart disease and would be unlikely to go to cardiac surgery, so are unlikely to be represented in the Deshmukh study. The differential gene expression changes between the AF/AF group and the AF/SR group may be because of the 2 phenomena: (1) the effects of AF-induced remodeling and (2) intrinsic factors that determine the vulnerability to long-term persistence, either by enhancing persistence in the AF/AF group or by reducing it in the AF/SR group. The ion-channel subunit changes in AF/AF patients indicate that AF-induced gene expression alterations are present. A hint to the existence of persistence-determining differences is provided by the fact that there are more gene expression differences between AF/AF and AF/SR than between AF/AF and no-AF: if AF/AF gene changes strictly consisted of AF-susceptibility factors plus those because of AF-induced remodeling, AF/AF would have to be more different from no-AF than from AF/SR. The presence of more differences between AF/AF and AF/SR than AF/AF versus no-AF suggests that some of the additional differences are in factors that determine the likelihood of remaining in AF long-term.

Relationship to Previous Work on the Molecular Determinants of AF Susceptibility and Persistence

The literature on AF mechanisms and associated molecular changes is vast and goes beyond what can be presented here. However, a few particularly pertinent observations will be highlighted. A recently published study showed that patients with paroxysmal AF are prone to delayed afterdepolarizations and triggered ectopic activity, apparently because of increased intracellular Ca$^{2+}$-load related to enhanced sarcoplasmic reticulum Ca$^{2+}$-uptake and ryanodine receptor dysfunction. Although long-standing persistent AF patients are also susceptible to delayed afterdepolarizations, the mechanisms and molecular basis are distinct from those in paroxysmal AF. The only gene expression changes in the Deshmukh database that might be associated with this type of activity were seen in the AF/AF group (upregulation of calmodulin, CALM3, and downregulation of junctophilin, JPH1), and the AF/SR group did not show any changes that clearly point to increased triggered activity. The lack of delayed afterdepolarization-promoting gene expression changes in AF/SR patients does not, however, exclude a molecular basis for triggered activity, as much of the molecular abnormalities underlying triggered activity in paroxysmal AF are post-translational.

Recent studies have also investigated the molecular determinants of the transition to long-standing persistent AF. Martins et al studied the progression to persistent and long-standing persistent AF in an atrial tachypaced sheep model. They noted a role for ionic remodeling (downregulation of $I_{Ca,L}$, $I_{Na}$, upregulation of $I_{K1}$; along with corresponding changes in ion-channel subunits) in persistence and interstitial fibrosis in long-standing persistence, both consistent with the characteristics of AF/AF patients in the Deshmukh article. Harada et al recently examined the response of atrial cardiomyocyte AMP-dependent protein kinase (AMPK) to metabolic stress and AF in a dog model. AMPK is a sensor-adaptor protein that is central in the response to metabolic stress, sensing increased AMP (which reflects reduced energy availability) and inducing changes that decrease metabolic requirements and increase energy liberation. Directly induced atrial cardiomyocyte metabolic stress or AF activate AMPK, which minimizes AF-promoting changes in $I_{Ca,L}$ and action potential duration. AF patients manifest biochemical changes suggesting metabolic stress. Patients with paroxysmal AF had increased fractional AMPK phosphorylation (activation) and those with long-standing persistent AF had reduced AMPK phosphorylation, suggesting that the level of AMPK activation might govern resistance to AF persistence. Interestingly, in the Deshmukh database, AMPK β-subunit gene expression is increased ≈2-fold in AF/SR versus no-AF, and reduced by ≈60% in AF/AF versus AF/SR, consistent with the notion that the activation of AMPK protects against long-term AF perpetuation.
Recent work in a mouse model examined the role of intracellular Ca\(^{2+}\) loading in the progression from atrial ectopy to paroxysmal through persistent AF.\(^\text{17}\) In mice with a mutation causing progressive AF, the authors found that sarcoplasmic reticulum Ca\(^{2+}\)-leak was enhanced in association with Ca\(^{2+}\)/calmodulin kinase-II-dependent hyperphosphorylation of the ryanodine receptor. The introduction of a nonphosphorylatable serine-to-alanine mutation at a critical Ca\(^{2+}\)/calmodulin kinase-II phosphorylation site on the ryanodine receptor suppressed Ca\(^{2+}\) leak, reduced structural remodeling, and prevented the development of persistent AF.\(^\text{17}\) It is conceivable that the prominent upregulation of CALM3 in AF/AF patients promotes Ca\(^{2+}\)/calmodulin kinase-II activation and AF-promoting remodeling.

**Concluding Comments**

A wealth of information is buried in the extensive database of differentially expressed genes from the Deshmukh study: this should prove useful for both data mining and hypothesis generation toward future investigation. The transcriptome constitutes only a small part of the molecular basis for the functional phenotype; future studies will need to address protein expression changes, post-translational modifications and epigenetic regulation. It will be important to obtain further characterization of the molecular determinants of AF persistence and vulnerability in better-characterized clinical populations, particularly with respect to the duration and frequency of AF episodes, as well as in well-defined animal models. Nevertheless, the Deshmukh article is an important contribution to our understanding of the molecular basis for clinical AF, and the authors are to be congratulated.

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