Human Atrial Ion Channel and Transporter Subunit Gene-Expression Remodeling Associated With Valvular Heart Disease and Atrial Fibrillation

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Background—Valvular heart disease (VHD), which often leads to atrial fibrillation (AF), and AF both cause ion-channel remodeling. We evaluated the ion-channel gene expression profile of VHD patients, in permanent AF (AF-VHD) or in sinus rhythm (SR-VHD), in comparison with patients without AF or VHD, respectively.

Methods and Results—We used microarrays containing probes for human ion-channel and Ca\(^{2+}\)-regulator genes to quantify mRNA expression in atrial tissues from 7 SR-VHD patients and 11 AF-VHD patients relative to 11 control patients in SR without structural heart disease (SR-CAD). From the data set, we selected for detailed analysis 59 transcripts expressed in the human heart. SR-VHD patients differentially expressed 24/59 ion-channel and Ca\(^{2+}\)-regulator transcripts. There was significant overlap between VHD groups, with 66% of genes altered in SR-VHD patients being similarly modified in AF-VHD. Statistical differences between the AF- and SR-VHD groups identified the specific molecular portrait of AF, which involved 12 genes that were further confirmed by real-time reverse transcription–polymerase chain reaction. For example, phospholamban, the \(\beta\)-subunit MinK (\(KCNE1\)) and MIRP2 (\(KCNE3\)), and the 2-pore potassium channel TWIK-1 were upregulated in AF-VHD compared with SR-VHD, whereas the T-type calcium-channel Cav3.1 and the transient-outward potassium channel Kv4.3 were downregulated. Two-way hierarchical clustering separated SR-VHD from AF-VHD patients. AF-related changes in L-type Ca\(^{2+}\)-current and inward-rectifier current were confirmed at protein and functional levels. Finally, for 13 selected genes, SR restoration reversed ion-channel remodeling.

Conclusions—VHD extensively remodels cardiac ion-channel and transporter expression, and AF alters ion-channel expression in VHD patients. (Circulation. 2005;112:471-481.)

Key Words: remodeling ■ ion channels ■ fibrillation ■ valves ■ atrium

Atrial fibrillation (AF), the most common cardiac arrhythmia, contributes substantially to cardiac morbidity and mortality.1 The cellular mechanisms underlying AF have been the subject of extensive studies both in human and in animal models (reviewed in reference 2). Ion-channel remodeling clearly plays an important role in the pathophysiology of AF, contributing to its initiation and perpetuation.2 At the functional level, decreased L-type Ca\(^{2+}\)-current (\(I_{Ca,L}\)) density is central to the shortening of the effective refractory period characteristic of AF.3 Downregulation of the transient outward current, \(I_{to}\), has also been reported,3–6 whereas inward-rectifier currents can be increased.3,7 Functional alterations in ionic currents have been associated with corresponding alterations in mRNA and ion-channel protein expression (see Brundel et al.,8 Van Wagoner and Nerbonne,9 and Nattel and Li10 for reviews).

Valvular heart disease (VHD) is a frequent clinical cause of AF.1,11 There is limited information available about ion-channel remodeling due to VHD, although VHD-related atrial dilation is associated with reduced \(I_{Ca,L}\) and \(I_{to}\).12

Previous studies of ion-transporter subunit remodeling associated with AF have been limited to the evaluation of a small number of candidate subunits believed to be important, with none examining >6 subunits. An additional limitation has been the fact that the independent contributions of AF and underlying heart disease have not been adequately addressed, because in studies of cardiac disease–related remodeling, many patients also have AF12

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and in studies of AF, many patients also have significant cardiac disease, often VHD. The present study was designed to use a genomic approach to address the following hypotheses: (1) Ion-channel subunit expression in patients with VHD differs from that in patients without VHD, reflecting ionic remodeling and (2) AF induces ion-channel remodeling beyond that induced by VHD alone, as reflected by different ion-channel subunit expression patterns in patients with AF and VHD compared with VHD patients in sinus rhythm (SR).

### Clinical Characteristics of Patients in Normal SR (VHD and SR-CAD Groups) and in AF at the Time of Cardiac Surgery

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age, y</th>
<th>Sex</th>
<th>Underlying Cardiac Disease</th>
<th>PMH</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR-VHD group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR-VHD1</td>
<td>76</td>
<td>M</td>
<td>AS</td>
<td></td>
</tr>
<tr>
<td>SR-VHD2</td>
<td>62</td>
<td>M</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td>SR-VHD3</td>
<td>80</td>
<td>M</td>
<td>AS</td>
<td>PV</td>
</tr>
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<td>SR-VHD4</td>
<td>72</td>
<td>M</td>
<td>AS</td>
<td>SM</td>
</tr>
<tr>
<td>SR-VHD5</td>
<td>52</td>
<td>M</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td>SR-VHD6</td>
<td>69</td>
<td>M</td>
<td>AR/AS</td>
<td>ST</td>
</tr>
<tr>
<td>SR-VHD7</td>
<td>78</td>
<td>F</td>
<td>AS</td>
<td></td>
</tr>
</tbody>
</table>

| AF-VHD group |        |     |                             |     |
| AF-VHD1 | 67     | M   | MR                          | CR  |
| AF-VHD2 | 70     | F   | MS                          | DG  |
| AF-VHD3 | 74     | M   | AS                          | AM+HCT |
| AF-VHD4 | 67     | M   | AS                          | RM  |
| AF-VHD5 | 76     | M   | AS/MS                       | FR  |
| AF-VHD6 | 72     | F   | MR                          | DT  |
| AF-VHD7 | 73     | M   | AS                          | DG  |
| AF-VHD8 | 73     | M   | AS                          | AT  |
| AF-VHD9 | 69     | M   | AS                          | AT  |
| AF-VHD10 | 77    | M   | AS                          | AT  |
| AF-VHD11 | 75    | M   | AS                          | AT  |

| SR-CAD group |        |     |                             |     |
| SR-CAD1 | 64     | M   | no                          | AC  |
| SR-CAD2 | 73     | M   | no                          | AT  |
| SR-CAD3 | 68     | M   | no                          | AT  |
| SR-CAD4 | 72     | M   | no                          | AT  |
| SR-CAD5 | 70     | M   | no                          | AT  |
| SR-CAD6 | 68     | M   | no                          | AT  |
| SR-CAD7 | 58     | F   | no                          | AT  |
| SR-CAD8 | 74     | M   | no                          | AT  |
| SR-CAD9 | 71     | M   | no                          | AT  |
| SR-CAD10 | 73   | F   | no                          | AT  |
| SR-CAD11 | 73   | M   | no                          | AT  |

LAD indicates left atrium diameter; PMH, previous medical history; ACE, angiotensin-converting enzyme; AT1, angiotensin II type 1; AS indicates aortic stenosis; MR, mitral regurgitation; AR, aortic regurgitation; MS, mitral stenosis; ST, sotalol; CR, carvedilol; AT, atenolol; AC, acebutolol; MT, metoprolol; BT, beta-blockol; DG, digoxin; DT, digitals; PV, pravastatin; SM, simvastatin; A0, atorvastatin; FR, furosemide; IN, indapamide; SP, spironolactone; AZ, alizide; AM, amiloride; HCT, hydrochlorothiazide; ID, isosorbide dinitrate; TT, trinitroglycerin; MS, molsidomine; AA, acetylsalicylic acid; AD, amolipine; CD, clopidogrel; CP, captopril; DL, diltiazem; EL, enamel; FE, felodipine; FL, flurbiprofen; FN, fluindione; FS, fosinopril; LS, losartan; LT, levothyrinone; NC, nicotine; PR, perindopril; RM, ramipril; VL, valsartan; +, present; and -, absent. Data in bold are presented as mean±SD.

**Methods**

**Human Samples**

For molecular biology studies, right atrial appendages (RAAs) were obtained during open-heart surgery from 7 VHD patients in SR (SR-VHD), 11 patients with VHD and permanent (>1 year) AF (AF-VHD), and 11 patients undergoing revascularization surgery for coronary artery disease (CAD), constituting the control group (SR-CAD). SR-VHD and AF-VHD groups were similar in terms of age, sex distribution, types of valve disease, left ventricular (LV) function, and atrial dimensions (the Table). Differences in drug therapy followed underlying heart disease: VHD patients received more
angiotensin-system suppressants and diuretics, which were less common in SR-CAD patients. SR-CAD patients commonly took β-blockers, statins, and Ca2+ antagonists. Ca2+ antagonists were prescribed for about half of the AF-VHD and none of the SR-VHD patients. To assess potential reversibility, we obtained data from an additional 6 patients with VHD and persistent AF, who were cardioverted and kept in SR for at least 1 month preoperatively (sinus-rhythm reversion [SRR-VHD] group). These patients were similar to other VHD groups in age (mean±SD, 74±1 years), types of valve disease (4 aortic, 1 mitral, 1 combined), LV ejection fraction (60±2%), and left atrial dimension (50±1 mm). Their drug therapy was also similar, except that they all received oral amiodarone. Finally, RAA samples were obtained from 7 SR-CAD, 6 SR-VHD, and 6 AF-VHD patients for functional studies. These additional patients showed comparable clinical characteristics to other groups. After excision, RAaS were immediately snap-frozen in LN2 and stored at −80°C.

RNA Isolation and Labeling
Total RNA was isolated with Trizol reagent (Life Technologies). RNA was DNase-treated (RNeasy fibrous tissue mini kit, Qiagen). The quality of isolated RNA was assessed by microelectrophoresis on polyacrylamide gels (Agilent 2100 Bioanalyzer). The absence of DNA contamination was verified by reverse transcription (RT)–negative polymerase chain reaction (PCR). A control pool was prepared, containing equal amounts of total RNA from each SR-CAD sample. RT was performed on this pool to obtain Cy3-labeled cDNA with the CyScribe cDNA postlabeling kit (Amersham Biosciences). For every SR-VHD and AF-VHD patient, 2 or 3 distinct RT reactions (depending on tissue availability) were performed to obtain Cy5-labeled cDNA. Each RT was processed independently.

Oligonucleotides and Microarrays
Fifty-mer, 5’ amino-modified oligonucleotide probes were synthesized at MWG Biotech AG. Lyophilized oligonucleotides were dissolved at 25 μmol/L in 1× spotting buffer A (MWG). The microarray stand was epoxysilane-coated slides. Oligonucleotide spotting was performed with the Lucidea array spotter (Amersham). Each microarray contained 4116 oligonucleotides (spotted in quadruplicate on each slide), including 315 targets for genes encoding human ion-channel subunits and proteins involved in calcium homeostasis.

Hybridization
Slides were treated with 50 mmol/L ethanolamine for oligonucleotide fixation. Each Cy5-labeled cDNA was mixed with an equal amount of Cy3-labeled control-pool cDNA, preincubated with yeast tRNA and polyA RNA (Gibco-BRL), and hybridized onto microarrays. After overnight hybridization, slides were washed with successively more stringent standard saline citrate solutions.

Forty-six independent hybridizations were performed in total: 2 for each SR-VHD patient and 2 or 3 for each AF-VHD patient. Hybridized arrays were scanned by fluorescence confocal microscopy (ScanArray 3000, GSI-Lumonics). Measurements were obtained separately for each fluorochrome at 10 μm/pixel resolution.

Microarray-Data Analysis
Fluorescence values were analyzed with GenePixPro 5.0. Raw data were processed with software (http://circdevise.nantes.insERM.fr/mad/madscan/login.php) developed internally.14 Low-quality spots were filtered, leaving only spots with valid expression values. Invariant genes were selected with the rank-invariant method.15 To normalize Cy3 and Cy5 values, a nonlinear regression method (Lowess fitness) was applied to invariant genes to calculate the correction for every spot of the microarray.16 Outliers were excluded by the median absolute deviation–modified Z test.17 To compensate for experimental variability and compare SR-VHD with AF-VHD data sets, expression-values of the 46 slides were scaled to the same median absolute deviation.18 Two statistical analyses were performed on the 4116-oligonucleotide data-points: (1) comparison of SR-VHD and AF-VHD groups based on data normalized to the SR-CAD control patient pool and (2) direct comparison of SR-VHD versus AF-VHD patients. Genes with statistically significant differential expression were identified with 1- and 2-class significance analysis of microarrays (SAMs)19 and linear models for microarray data (LIMMA).18 For SAM, genes with a q value <0.24% were selected. For LIMMA, genes with a Holm-corrected probability value <0.01 were selected. Genes were considered differentially expressed when they met both SAM and LIMMA criteria. For each gene, the median of replicate expression values was calculated. Oligonucleotides representing 59 genes encoding cardiac ion channels and calcium homeostasis proteins were retained for further study.

Two-way hierarchical agglomerative clustering was applied to the gene expression matrix consisting of 18 VHD patients and 59 selected genes. The input consisted of median replicate expression values (displayed as log-normal) for each gene and patient. Clustering with uncentered correlation with the CLUSTER program20 was visualized with Treeview software.

TaqMan Real-Time RT-PCR
First-strand cDNA was synthesized from 2 μg total RNA with Super Script III first-strand for RT-PCR (Invitrogen). Real-time PCR was performed with predesigned 6-carboxy-fluorescein (FAM)-labeled fluorogenic TaqMan probes and primers and 1× TaqMan universal master mix (Applied Biosystems). After 2 minutes at 50°C and 10 minutes at 95°C, 40 cycles of amplification were performed with the ABI PRISM 7900HT sequence-detection system (Applied Biosystems). Data were collected with instrument spectral compensation by Applied Biosystems SDS 2.1 software. Fluorescence signals were normalized to the housekeeping gene myosinisitol 1-phosphate synthetase A1 (ISYNA1). The comparative threshold-cycle (CT) relative-quantification method was used.21 For each patient, each gene was quantified in duplicate in 3 separate experiments. The values were averaged and then used for the 2−ΔΔCT×10 calculation, where 2−ΔΔCT corresponds to expression relative to ISYNA1. Statistical analysis was performed with ANOVA followed by Tukey’s test, with P<0.05 considered significant.

Western Blotting
Membrane proteins were extracted (n=6 in each group) and processed as previously described.22 Antibodies were obtained from Alomone Laboratories (Kir2.1 and Cav1.2) or Chemicon (Cx40).

IK1 and ICa Measurements
Human atrial myocytes were isolated23 and suspended in storage solution (mmol/L: KCl 20, KH2PO4, 10, glucose 10, potassium glutamate 70, β-hydroxybutyrate 10, taurine 10, EGTA 10, and albumin 1, pH 7.4). Whole-cell voltage-clamp IK1 and ICa, recordings were performed and analyzed as previously described.24 Cell capacitances averaged 89.4±8.8 (n=17) for SR-CAD, 94.3±7.6 (n=18) for SR-VHD, and 112.1±9.4 (n=22) pF for AF-VHD myocytes (P=NS).

Results
Gene Expression Changes of SR-VHD and AF-VHD Compared With SR-CAD Group
Figures 1 and 2 show ion-transport gene expression values in each VHD group. Results for calcium channels and regulators, Na+, K+–ATPase, and sodium-channel data are shown in Figure 1 and chloride, pacemaker, and potassium-channel genes and connexins in Figure 2. Results are given as percentage change from the control pool (SR-CAD group), with open circles representing individual patient values and horizontal lines representing medians. Red indicates statistically significant changes; black indicates results not signifi-
significantly different from the control pool. Of the 59 genes, 28 were unchanged in either SR-VHD or AF-VHD (indicated by gray boxes on the horizontal axis labels). Statistically significant changes in the same direction were observed for 16 genes (yellow boxes). For the 15 remaining genes, statistically significant changes were seen for only one of the VHD groups (green boxes). For no genes were there statistically significant changes in both groups that went in opposite directions. Thus, the SR-VHD group showed substantial changes from control, pointing to important remodeling of cardiac ion-transport genes in VHD and the need to consider VHD effects in the AF group.

To identify the AF-related molecular portrait, AF-VHD patients were compared with SR-VHD patients with 2-class statistical analysis. Among genes that were not significantly altered compared with control in either SR-VHD or AF-VHD alone, 2 genes (Kv4.3 and Cavα2δ2) showed a different distribution in SR-VHD compared with AF-VHD patients. Among genes that were significantly up- or downregulated congruently, MinK and Cavα2δ1 showed differential expression in SR-VHD versus AF-VHD patients. Among 15 genes that demonstrated significantly altered expression in 1 group only, 8 were differentially distributed in SR-VHD versus AF-VHD patients. In total, 12 genes showed differential expression in AF-VHD versus SR-VHD: the calcium-channel β-subunit Cavα2δ1 (CACNA2D1), phospholamban (PLN), the chloride-channel subunit CLCN6, the voltage-gated potassium channel β-subunit Kvβ1 (KCNA1B), the slow delayed-rectifier β-subunit MinK (KCNEL), the potassium-channel subunit MIRP2 (KCNEL3), and the 2-pore potassium-channel subunit TWIK-1 (KCNK1) were more strongly expressed in AF-VHD patients compared with SR-VHD, whereas the T-type calcium-channel α-subunit Cav3.1 (CACNA1G), the calcium-channel β-subunit Cavα2δ2 (CACNA2D2), inositol-triphosphate receptor 1 (ITPR1), the sodium-channel β-subunit Navβ2 (SCN2B), and the transient-outward potassium-current α-subunit Kv4.3 (KCND3) were less strongly expressed in AF-VHD than in SR-VHD patients.

Hierarchical Clustering Analysis Reveals AF-SR Differences

The results of unsupervised 2-way hierarchical clustering analysis are shown in Figure 3. Samples were grouped according to gene expression differences, ordering samples with the most similar expression patterns closest to each other and the most different patterns furthest apart. The hierarchical clustering analysis clearly separated SR-VHD from AF-VHD patients, displaying distinct transcriptional profiles of genes involved in cardiac electrical signaling. All 12 genes identi-
fied by 2-class analysis were included in discriminatory gene clusters (indicated at right of figure). Gene groups A and B were more strongly expressed in AF-VHD compared with SR-VHD, whereas the opposite was seen for group C. It should be noted that this hierarchical clustering indicates gene expression levels in SR-VHD relative to the AF-VHD group but not relative to the control group. Thus, some genes in groups A and B may show decreased expression versus the control pool, whereas others in group C may show increased expression (see Figures 1 and 2). Among the genes identified, 6 play potential roles in ionic currents that are modified in models of AF: Kv4.3 ($I_{Na}$), Kir2.1 ($I_{K1}$), MinK and MIRP2 ($I_{K}$), and Cav1.2/H1.2 and Cav1.2/H1.2 ($I_{Ca}$). Clustering was also attempted in relation to medication or type of VHD, and this analysis showed no detectable effect of these variables. When gene clusters A, B, and C were removed from the analysis, hierarchical clustering did not reveal gene expression differences between AF-VHD and SR-VHD samples (Figure 4), which intermingled rather than being distinctly separated as in Figure 3.

**Expression Assessment by Real-Time RT-PCR and Western Blotting**

Microarray data were further confirmed by TaqMan real-time PCR (Figure 5). The expression of the 12 genes showing characteristic expression in AF and clustering on hierarchical analysis was quantified on the RNA samples previously used for microarray experiments. In addition, we validated 2 genes that were not modified by either AF or VHD (SERCA2 and KvLQT1) and 4 genes that are classically altered by AF (NCX1, Cx43, Cx40, and Kir2.1). Transcripts were quantified with fluorescent probes with 100% PCR efficacy. This standardized method permits accurate quantification of transcript expression levels. Statistically significant differences between SR-VHD and SR-CAD groups are shown by asterisks (Figure 5), and differences between AF-VHD and SR-VHD groups are shown by pound symbols (#). Expression differences detected by real-time RT-PCR paralleled those identified by microarray. Of particular note are similar variations in inositoltrisphosphate receptor, phospholamban, CLCN6, Navβ2, Kvβ1, MiRP2, and Kv4.3. The expression level of Cav3.1 was too low to be reproducibly evaluated by RT-PCR and this is not shown in Figure 5. Finally, the 2 genes that were unchanged by either AF-VHD or SR-VHD showed no change by RT-PCR.

Western-blot experiments (Figure 6) were conducted for ion-channel subunits (Cav1.2, Kir2.1, and Cx40) selected on the basis of evidence for a pathophysiological role in AF. In agreement with gene expression data, Cav1.2 and Cx40 proteins were similarly expressed in SR-VHD and AF-VHD groups. Cav1.2 and Cx40 were significantly downregulated in SR-VHD versus SR-CAD. Kir2.1 was upregulated in the AF-VHD group only. Although changes were quantitatively similar for Cav1.2 and Cx40 mRNA and protein, upregula-
tion of Kir2.1 protein was more pronounced than Kir2.1 mRNA.

**Functional Correlation**

$I_{K1}$ recorded with a ramp protocol (Figure 7A and 7B) was significantly larger in AF-VHD than in SR-VHD or SR-CAD groups (Figure 7C). Peak $I_{Ca,L}$ (Figure 7B) was significantly smaller in AF-VHD than in SR-VHD (Figure 7C). $I_{Ca,L}$ was slightly, but not significantly, smaller in SR-VHD than SR-CAD.

**Reversibility of Ion-Subunit Changes**

To assess potential changes in ionic remodeling with SR restoration, we used quantitative RT-PCR to study ion-channel subunit expression after SRR. Figure 8 shows mRNA quantification for 13 selected genes (3 of which had been found downregulated, 4 upregulated, and 5 unchanged in the AF-VHD group). For each of the genes altered in the AF-VHD group, values for SRR-VHD were indistinguishable from those of SR-VHD, indicating their reversibility. Genes that did not differ between SR-VHD and AF-VHD were similarly unchanged for SRR-VHD.

**Discussion**

Although the link between VHD and AF is well recognized, the electrophysiological profile that distinguishes VHD patients who develop AF from those who do not remains unclear. Furthermore, there are relatively few data in the literature about the changes in ion-channel gene expression caused by VHD. The present study shows substantial remodeling in SR-VHD patients. It also shows that ion-channel subunit profiling discriminates SR-VHD patients from AF-VHD patients, and thus that there is a specific ion-channel subunit expression portrait for VHD patients with AF.

**Ion-Channel Expression Changes in Our Study Compared With the Literature**

In accordance with previous studies performed in dogs and humans, we found a clear reduction in L-type calcium-channel mRNA expression (Cav1.2 and Cav1.3) associated with a weak but statistically significant decrease in Nav1.5. The Cav1.2 differences were observed in both AF-VHD and SR-VHD patients and were confirmed at the protein level. However, functional data showed that $I_{Ca,L}$ was lower in AF-VHD than SR-VHD, which showed only a small trend to be lower than in SR-CAD. These data support the idea that the mechanism for $I_{Ca,L}$ reduction is complex and could involve altered regulation or other mechanisms distinct from mRNA and protein expression changes. Christ et al recently illustrated this complexity by showing that in AF patients, increased protein phosphatase activity may contribute to
impaired basal $I_{Ca,L}$. In both SR-VHD and AF-VHD groups, we observed upregulation of calcium-channel subunits Cav4.2 and Cav4.3. The physiological functions of these calcium-channel subunits are complex and their role in the heart is unknown, but they could contribute to determining L-type calcium-channel function in VHD and AF. Cav3.1 was upregulated in SR-VHD but unchanged in AF-VHD. Although there is some evidence of a role for T-type calcium-current density is not altered by atrial tachycardia–induced remodeling. Previous reports have indicated downregulation of Kv4.3 subunit expression in AF patients. Our initial microarray analysis did not show statistically significant Kv4.3 changes in either SR-VHD or AF-VHD groups. However, 2-class statistical analysis and hierarchical clustering indicated a significant contribution of Kv4.3 to the discrimination of SR-VHD from AF-VHD groups, with AF-VHD patients showing lower expression. In addition, real-time RT-PCR showed significant decreases in Kv4.3 expression in AF-VHD.

We are not aware of published studies of ion-channel subunit expression changes in VHD. Le Grand et al. showed that (1) L-type calcium-current density was decreased in patients with dilated atria, in agreement with our findings (Figure 1), and (2) transient-outward current was decreased, but to a lesser extent.

Our observation of Cx43 upregulation in AF is consistent with observations of Cx43 protein distribution by Elvan et al. and our observation of reduced Cx40 expression in AF agrees with the elegant experiments of Nao et al. These findings suggest that previously observed connexin-protein changes in AF may be due to transcriptional regulation. However, it should be noted that AF-related connexin expression changes have varied widely across studies, at least in part according to the model used. Upregulation of Cx45 may compensate for the decreases we observed in Cx40, but the role of Cx45 in the atria is unclear. NCX1 was upregulated in AF-VHD (albeit with pronounced interindividual variability) but not in SR-VHD. Upregulation of NCX1 protein has been reported in cardiac samples from AF patients, and NCX1 upregulation occurs in an animal model of AF related to congestive heart failure.

In agreement with previous findings, we observed down-regulation of Kir3.1 subunit expression in AF patients. Several studies have reported increased inward-rectifier current density in cardiomyocytes isolated from atrial tissues from patients with AF. The cardiac inward rectifier is carried not only by Kir2.1 channels but also by Kir2.2 and Kir2.3. In our study, Kir2.1 expression was unmodified in SR-VHD but slightly increased in AF-VHD patients, whereas Kir2.2 and Kir2.3 were upregulated in both SR-VHD and AF-VHD. RT-PCR, protein, and patch-clamp data showed a significant upregulation of Kir2.1 in AF-VHD, suggesting that (1) expression changes were slightly underestimated by the microarray technique, consistent with our previous findings, and (2) Kir2.1 is regulated posttranscriptionally. Previous reports have indicated downregulation of Kv4.3 subunit expression in AF patients. Our initial microarray analysis did not show statistically significant Kv4.3 changes in either SR-VHD or AF-VHD groups. However, 2-class statistical analysis and hierarchical clustering indicated a significant contribution of Kv4.3 to the discrimination of SR-VHD from AF-VHD groups, with AF-VHD patients showing lower expression. In addition, real-time RT-PCR showed significant decreases in Kv4.3 expression in AF-VHD.

Potential Significance of Our Observations
To our knowledge, this is the first study that used gene microarrays to evaluate ion-channel subunit-expression changes associated with AF and VHD. Thijsen et al. examined gene-expression changes in a goat model of AF with the differential display technique, but their approach did not address changes in ion-channel subunits. We obtained 2 principal general findings (1) that VHD produces important changes in cardiac ion-channel gene expression and (2) that discrete ion-channel subunit changes differentiate patients with VHD who develop persistent AF from those who do not. A number of the ion-channel and transporter subunits that are differentially affected in AF patients, including calcium-channel δ-subunits, inositoltrisphosphate receptors, CLCN6 chloride-channels, and a variety of potassium-channel β-subunits, have not previously been reported in AF-related ionic remodeling. Their significance will need to be addressed in further studies. The ion-channel changes differentiating AF-VHD from SR-VHD patients could be a result of AF, which was sustained for at least 1 year before surgery in the AF-VHD group, or could theoretically represent ionic current expression patterns favoring AF development. How-

Figure 4. Two-way hierarchical clustering applied to 44 selected genes. Removal of gene clusters A, B, and C in Figure 3 eliminates ability to discriminate AF-VHD from SR-VHD patients, illustrating importance of these clusters. Format as in Figure 3.
ever, our finding that the SRR-VHD group had a gene expression pattern like that of SR-VHD suggests that the specific AF-VHD profile is more likely a result of AF per se than of predisposing factors.

One important observation in our study is the overall similarity in ion-channel and transporter-expression changes in SR-VHD versus AF-VHD groups (Figures 1 and 2). This highlights the need to obtain a disease-matched control group for studies of gene expression changes associated with AF. Many previous studies of AF-associated remodeling have lacked such control groups, making it difficult to know whether the changes observed in the AF population were due to AF or the underlying heart disease.

**Study Limitations**

Overall, changes in the expression of ion-channel genes were small in amplitude, with the exception of changes in the L-type Ca$^{2+}$-channel Cav1.2 and Kir3.1 K$^+$-channel transcripts, which were decreased to less than half their control value. However, ion-channel gene expression is finely tuned. A 30% alteration in ion-channel mRNA could have a dramatic effect on cardiomyocyte electrophysiology if it translates into similar alterations at the levels of protein expression and current amplitude.

The AF-specific ionic remodeling described here was obtained in patients with >1 year of sustained arrhythmia. Different durations of AF and paroxysmal forms could produce different alterations. It is well known that the atria are heterogeneous tissues. Therefore, our data obtained in RAA samples may not accurately reflect alterations in the rest of the atria. Furthermore, ion-channel gene-expression changes in thoracic veins like the pulmonary veins, the ligament of Marshall, and the venae cavae may be different from changes in the present study and may be...
important in determining AF occurrence. The LA may better reflect the effects of VHD on ion-channel remodeling, and the impact of VHD on the right atrium, as shown here, may be an underestimate.

It would be practically impossible to confirm all of the gene expression changes we saw with Western blotting and functional studies. We were able to confirm alterations in Kir2.1, CaV1.2, and Cx40. In addition, our transcriptional data for RYR2, PLN, SERCA2, and CASQ2 agree with Western blot data obtained by Vest et al and El-Armouche et al (unpublished data). However, these data clearly do not bear on the rest of the transcripts studied.

A final limitation is the fact that our patients in AF were taking a variety of cardioactive drugs, which differed to some extent between SR-VHD and AF-VHD patients. An example is the higher incidence of aspirin treatment in the control group and of fluindone in the AF-VHD group. Although there was no obvious relation between specific drug treatments and
ion-channel expression pattern, as assessed with clustering, we cannot exclude some influence of these inevitable differences in drug therapy between patients in SR and those in AF.

The remodeling changes due to SR-VHD might be expected to parallel those in animal models of congestive heart failure and those associated specifically with AF-VHD to parallel those in models of atrial tachycardia remodeling. Although there are many similarities, there are also differences that could be due to species differences, discrepancies in the precise form of heart disease and its duration, and drug therapy effects in the clinical population. In addition, tachycardia-related remodeling is altered by concomitant heart failure, so that tachycardia-induced ionic remodeling in a pathological substrate may differ from what is seen in the normal heart, as in experimental paradigms.

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Remodeling of atrial ion-channel expression occurs in atrial fibrillation (AF) and may play an important role in its maintenance. Changes in ion-channel function have potentially significant implications for designing more effective and specific antiarrhythmic drug therapy for AF. In this study, we applied gene microarray technology to examine the expression of a wide range of cardiac ion channel and transporter genes in atrial tissues from patients with no atrial disease undergoing routine coronary-bypass surgery and valvular heart disease (VHD) patients in sinus rhythm (VHD-SR) or with longstanding AF (VHD-AF) undergoing valve-replacement surgery. We found substantial ion-channel gene-expression changes in both VHD-SR and VHD-AF, with roughly two thirds of remodeled genes being common to both; however, there was also a specific pattern of gene-expression change characteristic of VHD-AF, including several ion-channel genes not previously known to be remodeled in AF. Our study shows that VHD substantially affects ion-channel expression, emphasizing the importance of disease-matched controls for any AF-remodeling study, and that there is a pattern of ion-channel gene-expression change specifically related to AF. These findings have potentially important implications for understanding how VHD leads to AF, how AF alters cardiac ion-channel function, and how to design improved ion-channel–modulating antiarrhythmic drug targets for AF patients.
Human Atrial Ion Channel and Transporter Subunit Gene-Expression Remodeling Associated With Valvular Heart Disease and Atrial Fibrillation
Nathalie Gaborit, Marja Steenman, Guillaume Lamirault, Nolwenn Le Meur, Sabrina Le Bouter, Gilles Lande, Jean Léger, Flavien Charpentier, Torsten Christ, Dobromir Dobrev, Denis Escande, Stanley Nattel and Sophie Demolombe

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