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Contrasting Gene Expression Profiles in Two Canine Models of Atrial Fibrillation

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Abstract—Gene-expression changes in atrial fibrillation patients reflect both underlying heart-disease substrates and changes because of atrial fibrillation-induced atrial-tachycardia remodeling. These are difficult to separate in clinical investigations. This study assessed time-dependent mRNA expression-changes in canine models of atrial-tachycardia remodeling and congestive heart failure. Five experimental groups (5 dogs/group) were submitted to atrial (ATP, 400 bpm ×24 hours, 1 or 6 weeks) or ventricular (VTP, 240 bpm ×24 hours or 2 weeks) tachypacing. The expression of ≈21,700 transcripts was analyzed by microarray in isolated left-atrial cardiomyocytes and (for 18 genes) by real-time RT-PCR. Protein-expression changes were assessed by Western blot. In VTP, a large number of significant mRNA-expression changes occurred after both 24 hours (2209) and 2 weeks (2720). In ATP, fewer changes occurred at 24 hours (242) and fewer still (87) at 1 week, with no statistically-significant alterations at 6 weeks. Expression changes in VTP varied over time in complex ways. Extracellular matrix-related transcripts were strongly upregulated by VTP consistent with its pathophysiology, with 8 collagen-genes upregulated >10-fold, fibrillin-1 8-fold and MMP2 4.5-fold at 2 weeks (time of fibrosis) but unchanged at 24 hours. Other extracellular matrix genes (eg, fibronectin, lysine oxidase-like 2) increased at both time-points (≈10, ≈5-fold respectively). In ATP, mRNA-changes almost exclusively represented downregulation and were quantitatively smaller. This study shows that VTP-induced congestive heart failure and ATP produce qualitatively different temporally-evolving patterns of gene-expression change, and that specific transcriptomal responses associated with atrial fibrillation versus underlying heart disease substrates must be considered in assessing gene-expression changes in man. **(*Circ Res*. 2007;100:0-0.)**

Key Words: arrhythmia ■ remodeling ■ genomic

Atrial fibrillation (AF) is the most common sustained cardiac rhythm disorder, and with the aging of the population both the prevalence and economic impact of AF are increasing progressively.[1] Although the mechanistic basis of AF remains incompletely understood, active research promises to provide new insights that may lead to improved therapeutic options.[2,3]

A variety of animal models have been used to assess AF pathophysiology under controlled conditions. Atrial tachyarrhythmias, including AF itself, alter atrial electrophysiology in ways that promote AF vulnerability.[4–6] Experimentally-induced congestive heart failure (CHF) also creates a substrate for AF maintenance, but by quite different mechanisms.[7] The atrial-tachycardia remodeling paradigm shows prominent changes in ion-channel function that lead to action-potential abbreviation and the promotion of atrial reentry.[8,9] CHF-induced ionic-current changes do not promote reentry but may favor ectopic-impulse formation,[10] and CHF-induced fibrosis promotes reentry by interfering with intraatrial conduction.[7]

The molecular basis of AF remains unclear. Gene microarray technology permits large-scale analysis of cardiac gene-expression changes, and has been applied to compare AF patients with those in sinus rhythm. Expression profiling has pointed to several AF-related gene-expression changes,[11–15] including alterations associated with oxidative stress,[11] a ventricular-like expression signature[14] and changes in ion-transporters.[15] A limitation of this type of clinical gene-expression study is that it is very difficult to differentiate between AF-promoting changes caused by AF and those because of underlying cardiac disease. The analysis is further complicated by systematic inter-group differences in drug therapy, atrial size, and other cardiac variables.

Animal models of AF allow for greater control over study conditions and permit observations of the time course of any

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alterations. A human DNA microarray containing 6,035 cDNA probes applied to a porcine model of AF pointed to changes in myosin light chain-2 expression.[16] DNA microarrays with probes for canine-gene transcripts have recently become commercially-available. We designed the present study to analyze changes in canine cardiac gene-expression in 2 AF models: atrial-tachycardia remodeling induced by atrial tachypacing (ATP) and CHF-related remodeling produced by ventricular-tachypacing (VTP). Assessments were initially obtained at 2 time-points in each model: early after the onset of tachypacing (24 hours) and at a time of near steady-state remodeling (1 week for ATP,[8] 2 weeks for VTP[17]). After initial studies showed that AF duration increases were smaller in 1-week ATP dogs versus 2-week VTP dogs, we added another group subjected to 6-week ATP.

Materials and Methods

Because of manuscript-length limitations, we summarize only key methods in this section. Detailed methods are provided in the online data supplement available at http://circres.ahajournals.org

Animal Model

These methods followed previous publications.[6,7,10,18,19] Experiments were performed in male mongrel dogs weighing 25 to 32 kg. In the initial series of experiments, 5 groups (n=5/group) were studied. Two groups were subjected to VTP, for 24 hours or 2 weeks. A unipolar ventricular-pacing lead was inserted into the right-ventricular apex under 1.5%-halothane anesthesia and attached to a pacemaker in the neck programmed to 240 bpm. Two other groups were subjected to ATP, for 24 hours or 1 week. These dogs were instrumented with a unipolar right-atrial (RA) lead attached to a pacemaker that captured the atrium at 400 bpm and a ventricular-pacing lead attached to a demand-pacemaker programmed to 80 bpm. Complete AV-block was induced by radiofrequency-ablation. A final group of VTP-sham control animals was handled identically to 24-hour VTP-dogs, but their pacemaker was not activated.

A second series of concurrent experiments was performed in 3 additional groups of dogs (n=5/group): because 1-week ATP proved not to promote AF as much as 2-week VTP, we added a 6-week ATP group, and to test for potential intervention-related differences we concurrently studied VTP and ATP shams. The VTP-shams were prepared as described above. The ATP-shams were prepared and handled identically to the 24-hour ATP-dogs, but their atrial pacemakers were not activated. VTP-shams were included in both experimental series because they were the primary control group for all analyses. For biochemical analyses in each series, experimental and sham animals were handled concurrently, with DNA extraction and microarray processing performed on the same days with the same reagents and on the same batches of microarrays to minimize variability. After preparation periods, dogs were anesthetized (morphine, 2 mg/kg s.c.; α-chloralose, 120 mg/kg i.v. load; 29.25 mg/kg/hr maintenance infusion) and ventilated. In vivo measurements were obtained and an isolated-cardiomyocyte preparation was snap-frozen for subsequent analysis.

In Vivo Measurements and Cell Isolation

A median sternotomy was performed and Teflon-coated stainless steel electrodes were hooked into the RA appendage (RAA) for electrophysiological measurements. RAA effective refractory period (AERP) was measured at various basic cycle lengths (BCLs). Mean AF duration (DAF) was measured in each dog with multiple AF inductions as previously described.[6–8] Left-ventricular end-diastolic pressure was measured at the end of each experiment. Animals were euthanized, hearts removed and placed in Tyrode solution equilibrated with 100% O_2 for dissection. The left atrium (LA) was perfused via the left circumflex coronary artery and cell-isolation performed via collagenase digestion.[18,19] Electrophysiological measurements were obtained in the RAA and cell isolation/biochemical analysis performed with LA tissue because in our previous experience tissue trauma caused by atrial manipulation for electrophysiological measurement can affect biochemical determinations.

Canine Genome Microarrays

The microarrays we used (Affymetrix GeneChip Canine Genome Array) are high-density oligonucleotide arrays (11-μm spots) containing 23,836 25-mer probe-sets detecting 21,700 transcripts. For details, see the online data supplement available at http://circres.ahajournals.org.

RNA Extraction, Quantification, and Processing on Arrays

RNA was extracted into chloroform from Trizol-immersed cell pellets (for details, see online data supplement). RNA was quantified and assessed for purity by measuring optical density at 260 nm and 280 nm. The quality was also verified by running samples on 2.5% agarose gels. Samples with OD ratio 260/280 nm >1.8 were selected for microarray processing.

For processing on microarrays, 10 μg of total RNA was used. The target cRNA derived from each sample was verified for quality on Agilent RNA 6000 nano-chips and fragmented cRNA was hybridized to the microarray. The chips were stained and washed with the GeneChip Fluidics Station 450 and visualized on an Affymetrix GeneChip Scanner 3000.

Statistical Analysis of Microarray Data

The microarray expression data were analyzed using a combination of algorithms. We first applied the invariant set normalization method[20] in dChip, which corrects for interarray differences in brightness. To integrate each gene's probe intensities into one value representative of gene-expression, we used dChip to calculate the model based expression index.[21] dChip was set to use only the intensities of the perfect match probes as well as detect single, probe, and array outliers. After formulating the gene-expression values, we used significance analysis for microarrays (SAM)[22] to detect differentially-expressed genes, accepting only genes with a q-value <1.

For genes without Affymetrix annotations, we used BLAST to find mammalian-sequence homologies with an E-value $<10^{-4}$. To enhance the annotations with functional information, we used Affymetrix's human to canine microarray comparisons to map canine genes to their human equivalents. With the human equivalents, we queried the Gene Ontology (GO) database for functional information. Genes not identified by this process were classified by literature search.

Western-blot Analysis

The expression of selected genes was verified at the protein level by Western blot. Protein was extracted from isolated-cardiomyocyte pellets. Equal amounts of protein (100 μg/sample) were separated by SDS-polyacrylamide gel electrophoresis, transferred and then blotted with antibodies listed in supplemental Table I. Bands were quantified and expressed as a ratio over the corresponding VTP-sham sample in the same gel, relative to GAPDH band-intensity on the same samples.

Real-time RT-PCR

Microarray-based expression ratios were confirmed with real-time RT-PCR for 18 selected genes. First-strand cDNA was synthesized from 2 μg of total RNA. Online PCR was performed with FAM-labeled fluorogenic TaqMan probes and primers. The fluorescence signals were normalized to results with 18S-ribosomal RNA and analyzed with the comparative-threshold cycle (Ct) method. Each gene was quantified in duplicate for each sample. Forward and reverse primers and TaqMan probe-sequences are provided in supplemental Table II.

Statistical Analysis

Data are expressed as means±SEM. Comparisons among group means (Tables 1 and 2 and supplemental Tables V and VI) were performed

TABLE 1. Hemodynamic and Electrophysiological Changes in First Series of Dogs.

	VTP-Sham	24-Hour VTP	2-W VTP	24-Hour ATP	1-W ATP
SBP, mm Hg					
Systolic	136±7	109±16	108±15	133±20	129±10
Diastolic	90±5	68±9	70±12	75±14	77±8
LVP, mm Hg					
End-diastolic	1±1	5±2	16±5**	2±2	2±2
AERP, ms					
BCL					
360	122±11	119±8	136±12	113±18	70±5**
300	125±10	117±9	133±13	120±12	71±5**
250	124±13	113±11	129±12	119±7	76±7**
200	117±7	108±10	121±13	112±5	75±7**
150	98±7	96±10	102±7	98±8	76±9*
DAF, s	39±25	300±360	837±436*	15±8	339±384

SBP=systemic blood pressure; LVP=left-ventricular pressure; BCL=basic cycle length. *, **$P<0.05$, 0.01 vs VTP-sham.

with one-way analysis of variance (ANOVA) followed by Dunnett's test for individual-mean comparisons relative to control (VTP-sham). A two-tailed $P<0.05$ was considered statistically-significant.

Results

Hemodynamics and Electrophysiology

Consistent with previous studies,[7,10] VTP-dogs showed increased left ventricular end-diastolic pressure and no significant AERP changes (Table 1). ATP-dogs were hemodynamically similar to sham controls, but as in previous work[4–6] showed substantial AERP decreases and loss of rate-adaptation at 1 week (Table 1). DAF increased progressively in both models, with changes reaching statistical significance at 2 weeks in VTP-dogs (Table 1) and at 6 weeks in ATP-dogs (Table 2). Although the ATP-induced AERP changes (decreased AERP and loss of AERP rate-adaptation) reached a maximum at 1 week (Table 1) and did not progress further at 6 weeks (Table 2), AF duration continued to increase between 1 and 6 weeks and statistically significant increases relative to baseline were achieved only at 6 weeks. There were no statistically-significant differences between ATP-shams and VTP-shams (Table 2).

TABLE 2. Hemodynamic and Electrophysiological Changes in Second Series of Dogs

	VTP- Sham	ATP- Sham	6-w ATP
SBP, mm Hg			
Systolic	133±14	134±11	121±3
Diastolic	85±11	74±7	65±1*
LVP, mm Hg			
End-diastolic	4±2	4±4	1±2
AERP, ms			
BCL			
360	124±4	120±2	86±12**
300	120±3	121±3	88±10**
250	124±13	113±11	87±9**
200	127±10	121±7	88±9**
150	108±7	102±6	83±8*
DAF, s	87±39	21±6	997±324**

SBP=systemic blood pressure; LVP=left-ventricular pressure; BCL=basic cycle length. *, **, $P<0.05$, 0.01, vs VTP-sham.

Microarray Findings

Figure 1 shows all mRNA-expression levels in the initial series of dogs, with the mean value for each transcript probe-set plotted against mean VTP-sham expression. Values indicated by blue points are not significantly different from sham-values, whereas red points indicate statistically-significant changes. In ATP-dogs, 242 probe-sets showed significant changes at 24 hours and 87 at 1 week. VTP-dogs showed significant changes for 2209 probe-sets at 24 hours and 2720 at 2 weeks. Corresponding results for the second series of dogs are shown in Figure 2. No statistically-significant gene-expression differences were observed between 6-week ATP-dogs and VTP-shams, nor between ATP-shams and VTP-shams. A full listing of relative expression values for all ATP and VTP groups are provided for each transcript probe-set with statistically-significant changes in supplemental Table III. Almost all (94%) of the significantly-changed genes in ATP-dogs lie below the black line of identity and are therefore under-expressed. For VTP-dogs, large numbers of genes lie on either side of the line of identity (53% underexpressed; 47% overexpressed).

Of the differentially-expressed genes in ATP-dogs, 55 of the downregulated and none of the upregulated values were common to both 24-hour and 1-week time-points. These 55 downregulated values make up more than 72% of the downregulated results at 1-week ATP- thus, most of the differentially-expressed genes at 1 week are also downregulated at 24 hours. The ATP-pattern indicates a stronger early response, with decreasing numbers of significantly-altered genes over time. Unlike ATP, the VTP model had 25% more significantly-altered expression values at the later time-point. There are 336 upregulated and 567 downregulated transcript

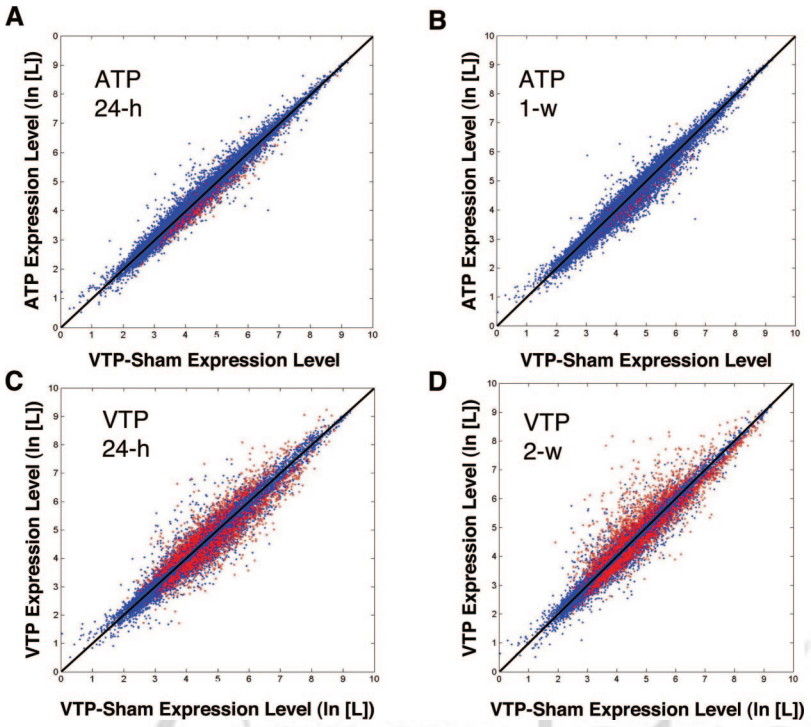

Figure 1. Overall changes in mRNA expression compared with sham in 24-hour ATP (A), 1-week ATP (B), 24-hour VTP (C) and 2-week VTP (D) samples. The absolute expression, in $\log_e(L)$, where L=mean sample-luminescence, for each intervention-group transcript probe-set is plotted as a function of the corresponding value for the VTP-sham group and represented as a single point. Blue points are not significantly different from sham; red points are significantly different. If no changes in mRNA-expression occurred, all points would fall on the black line of identity.

probe-sets common to both time-points, constituting 41% and 33% of the significantly-altered 24-hour and 2-week values respectively. Thus, more than half of the genes differentially-expressed in VTP at each time point are unique to that time point. For the transcript probe-sets common to both time points, a statistically-significant fraction (61%) was less altered at the 2-week time point.

Figure 3 shows the functional categories of genes that are significantly up- and downregulated in ATP and VTP-dogs. In ATP-dogs, the categories with the most genes altered are DNA/RNA synthesis/degradation and signal transduction. Almost all significantly-changed genes were downregulated. For all but the ribosomal-gene category, more genes were changed at 24 hours (black bars) than 1 week (white bars). The gray bars, representing genes changed at both 24 hours and one week, show that for most groups all genes changed at 1 week were also significantly altered at 24 hours. Several gene-categories (apoptosis, extracellular matrix (ECM), and transport) have no representation at 1 week. In contrast, for VTP-dogs most of the functional groups show unique 24-hour and 2-week responses, with overlap representing less than half the total response. Approximately as many values in each group represent upregulation as downregulation.

To evaluate whether gene-groups respond uniformly to each intervention or whether specific gene-groups change differentially, we calculated the percentage-change relative to sham in each dog for each significantly-altered transcript probe-set and ranked all changes from largest to smallest, with 1 being the most-changed gene and the highest rank-number the least-changed gene. We then plotted for each gene-group the fraction of its ranks that fell within each cohort of genes as ranks increased by integer from 1 (the most changed expression-value) to the least-changed value. To assess statistical significance, we calculated the sum of ranks for each functional gene-group and then randomly reassigned expression ratios to groups. For each permutation, we calculated the sum of ranks for the random groups and compared them to the originals. This process was repeated 100,000 times, and those gene-groups whose sum of ranks were lower/higher than the random groups more than 97.5% of the time (2-tailed $P<0.05$) were considered to have significantly larger/smaller expression-changes compared with overall behavior. Results following average behavior are shown by black lines; groups deviating significantly from average are shown by blue or red lines for larger or smaller changes respectively (Figure 4). For 24-hour VTP-dogs (Figure 4A), genes associated with metabolism, ECM, and cell structure/mobility showed the largest changes. At 2-week VTP (Figure 4B), the same functional categories show larger-than-average changes, along with immunity/coagulation genes. At both time points, ribosome-associated genes showed smaller-than-average changes, but DNA/RNA synthesis/degradation genes only deviated from average at 2 weeks. The analyses are less clear for ATP-dogs (supplemental Figure I), because of the much smaller number of significantly-changed genes. At 24 hours, immunity/coagulation and metabolism genes occupy significantly higher ranks than other groups, whereas at 1 week no groups deviate significantly from average responses. Supplemental Table IV shows detailed values and distribution properties from these analyses.

Real-time RT-PCR Results

Figure 5 compares the expression levels of selected genes as determined by microarray and real-time RT-PCR methods in the first series of dogs. The genes were selected to include genes believed to be of pathophysiological significance, as well as genes with overexpression, underexpression and no apparent expression change. Overall, there was a strong linear

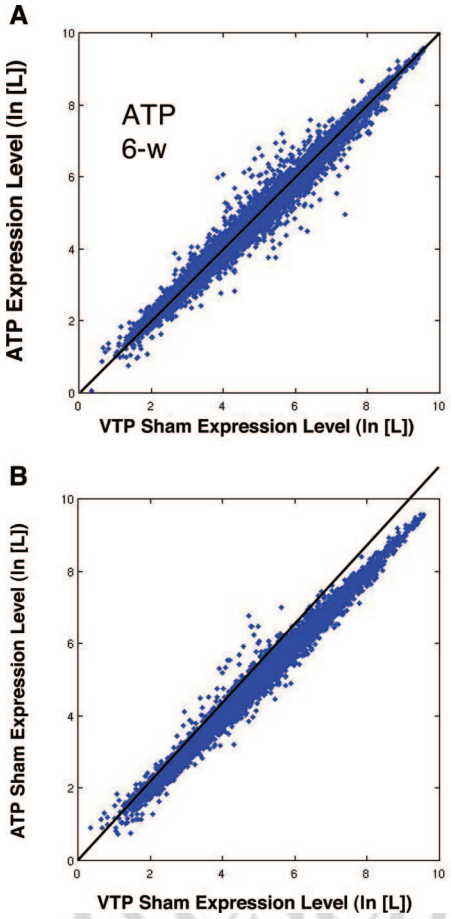

Figure 2. Overall mRNA-expression comparisons between (A) 1-week ATP versus VTP-sham (B), ATP-sham versus VTP-sham. Format as in Figure 1.

correlation between results with the 2 methods (R^2=0.96). Because most of the values were concentrated within the 0 to 5-fold change range, this section of the graph is expanded at the left for better resolution. Detailed results are presented in supplemental Table V. There is generally close agreement between the independent determinations of mRNA expression by the 2 methods. Of 36 sample-sets showing statistically-significant changes by microarray, 22 (61%) show statistically-significant changes of the same order and direction by RT-PCR. For the 14 sample-sets with significant changes by microarray and nonsignificant changes by RT-PCR, 11 (79%) show changes of the same direction and order with both methods.

Western-blot Results

Figure 6 shows typical blots for the 9 gene products selected for Western-blot analysis. Supplemental Table VI presents the mean results of Western-blot analyses, along with the corresponding results from microarray analysis. Statistical congruence was observed for 28 of 36 sample sets: 24 sample-sets (shown in green in supplemental Table VI) did not change significantly by either gene-chip or Western blot, and 4 sample-sets (shown in yellow in supplemental Table VI) changed significantly in the same direction. For 5 of the remaining 8 sample-sets (pink in supplemental Table VI), changes were statistically significant for only 1 of gene-chip or Western blot, but were in the same quantitative direction. For 3 sample-sets (cathepsin L in 2-week VTP and cathepsin S in 24-hour and 2-week VTP dogs), statistically-significant increases or decreases of the order of 30% to 50% were seen in one measurement and either directionally-discrepant or no change was observed with the other. There was thus good general agreement between changes in protein expression and changes in mRNA-expression, although some quantitative differences were clearly present (eg, for collagen-III, KChIP2 and IL1-RA).

Discussion

We have analyzed changes in canine atrial mRNA expression induced by atrial-tachycardia remodeling and ventricular-tachypacing induced heart failure over time. The results highlight major differences in the molecular basis of these 2 atrial arrhythmogenic-remodeling paradigms and indicate important time-dependent evolution of gene-expression changes.

Relationship to Previous Findings Regarding Gene-expression Profiles in AF

Several gene-microarray studies have been performed in AF patients. Kim et al found upregulation of pro-oxidant and downregulation of antioxidant genes.[11] Investigators subsequently found 33 genes with >50% upregulation and 63 with >50% downregulation,[12,13] with changes in genes related to cell signaling, inflammation, oxidation and cellular respiration.[13] Barth et al found that the human atrial transcriptome changed to a ventricular-like pattern in AF-patients.[14] One limitation of these studies was the difference in heart disease between AF-patients (valve disease, often with cardiac hypertrophy, dilation and/or dysfunction) compared with sinus-rhythm controls (coronary-artery disease with well-preserved ventricular function). It is therefore difficult to separate changes because of AF from underlying disease-related remodeling. We recently analyzed AF-related transcriptome remodeling in heart-disease matched patients with AF versus sinus rhythm.[15,23] Most gene-expression changes were attributable to underlying heart disease: ≈2/3 of ion-channel gene-changes[15] and >90% in the complete transcriptome[23] occurred in both sinus-rhythm and AF patients.

Much less information is available from animal models of AF. Changes in cellular structure, metabolism, gene-expression regulation and differentiation genes were observed in a goat model.[24] In a porcine atrial-tachypacing model, 387 genes were altered.[16] In neither model was ventricular-rate controlled, so a contribution of tachycardia-induced cardiomyopathy cannot be excluded.

Here, we used canine-specific microarrays to study changes with ≈21,000 transcript probe-sets over time in ATP and VTP-dogs. Our results show striking differences in the quantity, magnitude and types of gene-expression changes induced by the 2 interventions. Whereas atrial-tachypaced dogs primarily showed decreasing numbers of transcript-expression changes over time, VTP-dogs displayed complex

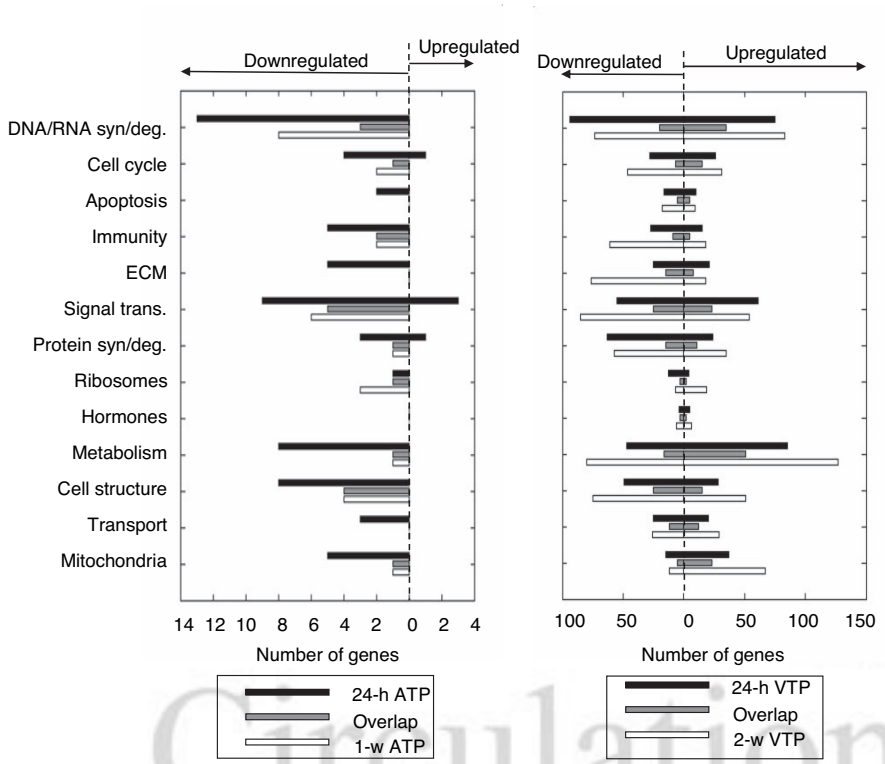

Figure 3. Number of transcript probe-sets in each functional group which was significantly down- or upregulated by atrial-tachycardia remodeling (left) or ventricular-tachycardia remodeling (right). "Overlap" refers to the number of values that were significantly affected in the same direction at both 24 hours and 1 week.

temporal evolution with some transcripts becoming less affected over time and others more affected. Particularly striking were changes in ECM-gene expression, with relatively small changes in most genes at 24 hours and very large changes at 2 weeks (eg, 8 collagen-genes upregulated >10-fold, fibrillin-1 8-fold and MMP2 4.5-fold; supplemental Table III). However, fibronectin was >10-fold and lysine oxidase-like (LOXL)-2 was ≈5-fold upregulated at both 24-hour and 2-week time-points. ECM genes were virtually unchanged in ATP-dogs, with small (≈20% to 30%) decreases in 3 collagen genes at 24 hours and no significant ECM-gene changes thereafter.

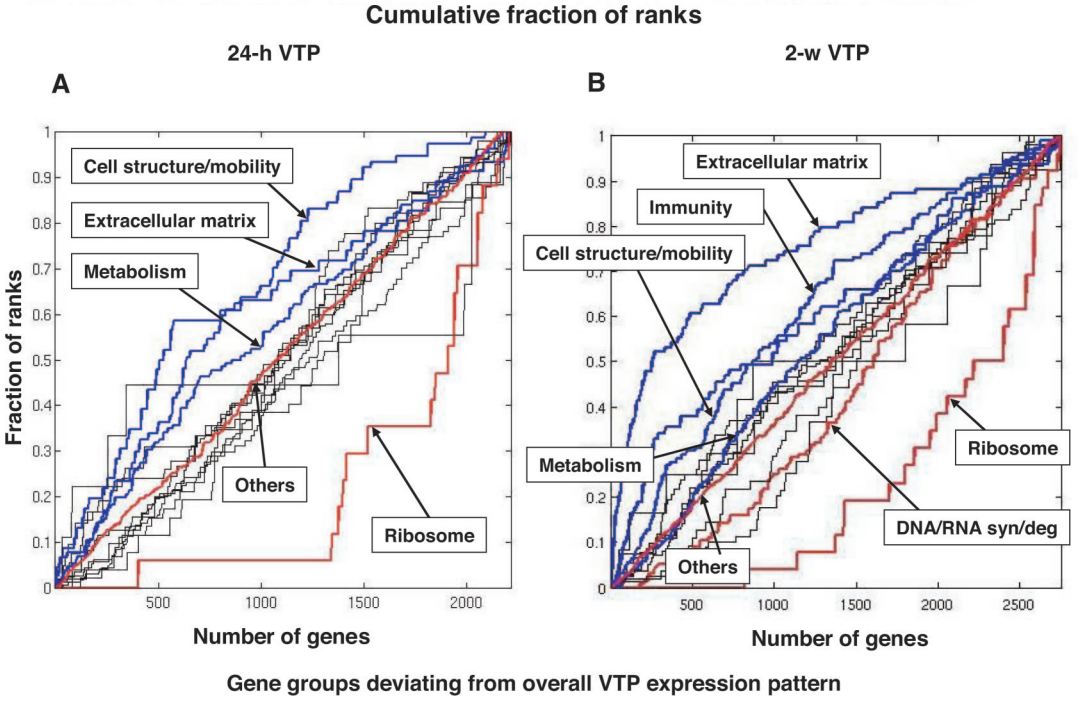

Figure 4. Cumulative fraction of ranks for each of the functional gene groups in VTP-dogs (for discussion of method, see text). Functional group curves in blue showed expression-ranks significantly greater than that of overall genome indicating larger-than-average changes; functional groups in red showed expression-ranks that were significantly less, indicating smaller-than-average changes.

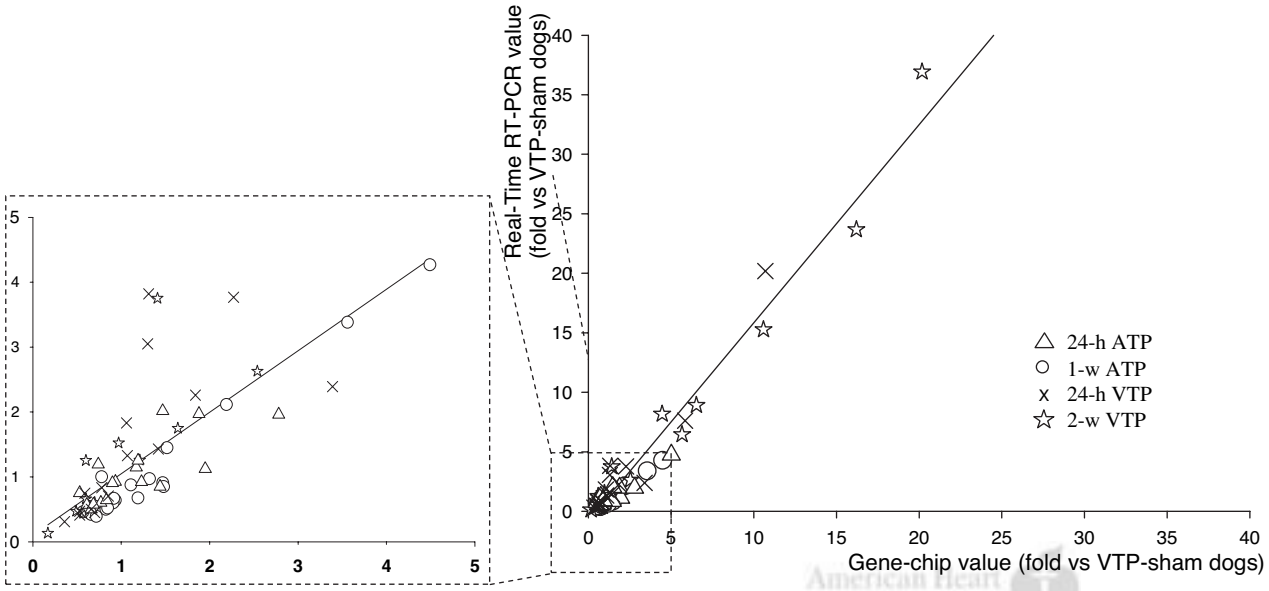

Figure 5. Correlation between expression changes by real-time PCR versus microarray. Regression lines are shown.

Relevance to Mechanisms of AF-related Remodeling

Ventricular tachypacing-induced CHF produces structural and ionic remodeling resembling the substrate for chronic AF in man.[3,7,10] Our results provide extensive new information about the nature and number of atrial gene-systems affected over time during the evolution of CHF. The prominence of changes in ECM genes, particularly those associated with collagen production, is consistent with the fibrosis that appears central to arrhythmogenesis.[25,26] Collagen-associated mRNA expression is dramatically increased at 2-week VTP, when fibrosis approaches maximum, and is much less affected at 24 hours, when fibrosis has not yet appeared.[17] Early-phase reactive ECM genes, such as α_1-antitrypsin and fibronectin (activity increased ≈5 and ≈10-fold at 24-hour VTP), may be involved in early changes leading to fibrosis. Altered regulation of genes involved in metabolism and cellular contraction are consistent with energy-saving adaptations.

To pursue the gene-response analysis, we considered genes whose protein-products interact with TGFβ, in view of evidence for a potentially-important role of TGFβ in AF-related fibrotic remodeling.[27,28] We identified all proteins in the Human Protein Reference Database that interact with TGFβ or a TGFβ-interacting protein. For 383 proteins identified, we used BLAST to find corresponding probe-sets on our microarray. Of 214 genes whose expression could be measured, 85 were differentially expressed in VTP-dogs (supplemental Figure II). Interestingly, connective-tissue growth factor (CTGF) gene-expression was enhanced by

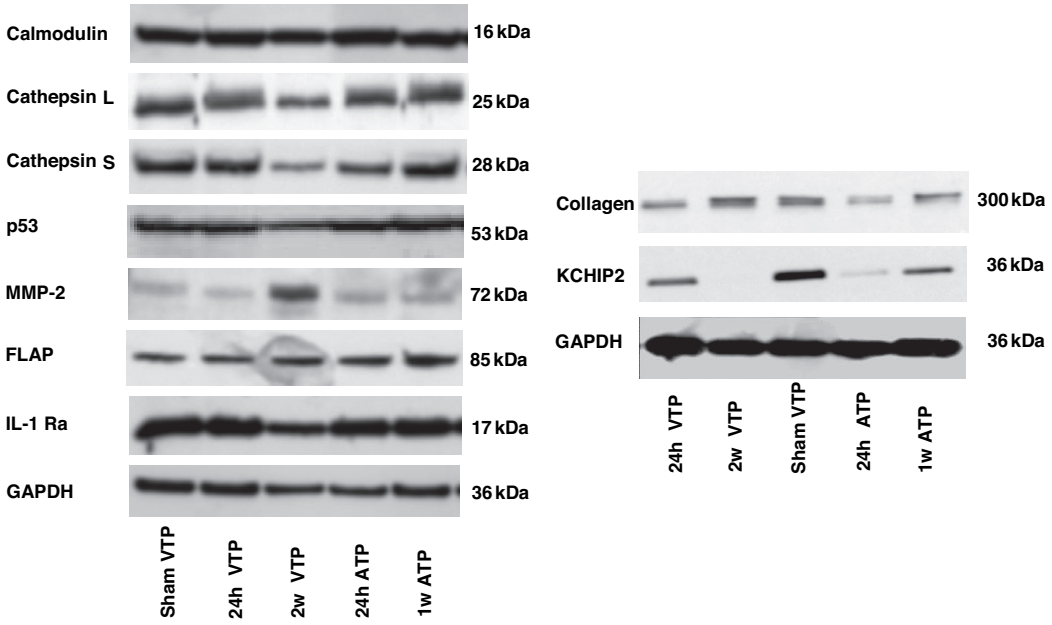

Figure 6. Examples of Western blots for 9 proteins studied to compare protein versus mRNA expression changes.

24-hour VTP. CTGF is upregulated by angiotensin 2,[29] TGFβ1,[30] or alterations in the cytoskeleton.[31] CTGF promotes fibrosis in pathological conditions by blocking a negative TGFβ-feedback loop mediated by Smad 7 signaling, allowing continued TGFβ-related activation.[32] The addition of CTGF to primary mesangial cells induces fibronectin production, cell migration, and cytoskeletal rearrangement.[33] Fibronectin expression is enhanced by 24-hour VTP and fibronectin interacts with a wide range of TGFβ-related products of genes altered in VTP (supplemental Figure II). Thus, CTGF is an interesting potential candidate for a significant role in VTP-related remodeling. Further exploration of this and other networks identified by the rich genomic data obtained in the present work is indicated, but goes beyond the scope of this study. The very large number of genes affected by VTP points out the potential pitfalls in assessing a small number of selected genes without understanding the gene-response background against which such changes occur.

In contrast to VTP-remodeling, ATP-remodeling is associated with preserved tissue architecture and a predominance of ionic remodeling.[7,9,34] The virtual absence of ECM-gene changes in the ATP data set is consistent with this structurally-benign remodeling. We observed relatively limited changes in ion-channel genes, despite the importance of ion-channel alterations in ATP-induced AF promotion.[3,9,34] This may reflect important post-transcriptional mechanisms,[35,36] but likely also reflects relatively low-level expression of ion-channel genes that makes it difficult to differentiate ion-channel gene-expression changes from background noise in a pan-genomic microarray. To assess ion-channel subunit mRNA changes accurately requires specialized microarrays[15] that are presently unavailable for the dog. The decreasing number of gene-expression changes that occurred over time with ATP-remodeling suggests a time-related reduction in the stimuli for gene-expression change, consistent with atrial adaptation to the stress of ATP.

Potential Significance.

Clinical gene-microarray studies in AF are limited by a range of factors (eg, underlying cardiac disease, drug therapy, duration of AF, etc) varying within the population. Animal models permit the assessment of transcriptomal changes under controlled duration and nature of atrial-remodeling stimuli. The present study is the first assessment of mRNA-expression remodeling in animal AF models to use species-specific microarrays. In addition, it is the first to compare atrial-transcriptome remodeling because of atrial tachycardia per se with remodeling caused by an AF-promoting cardiac condition (CHF) and the first to study the evolution of gene-changes over time. Our results illustrate the importance of considering underlying disease-related gene-expression changes in AF populations. They also clearly contrast the relatively modest mRNA-expression changes caused by atrial tachycardia with the extensive alterations induced by CHF.

Potential Limitations

We selected analysis time-points based on evidence that early-phase VTP-induced atrial changes peak at 24 hours,[17,27] important atrial electrophysiological remodeling occurs with ATP at 1 week[8,31] and with VTP at 2 weeks.[17,27] Time-points additional to the ones we used might be interesting to examine. We do not know whether cardiac mRNA-level alterations were because of changes in synthesis or degradation. The studies necessary to resolve this question are not presently feasible at the scale that would be needed for the large number of genes we studied. We used an isolated-cardiomyocyte preparation for which we previously found very little contamination by other cell-types, but we cannot totally exclude contributions from noncardiomyocyte cell populations. Atrial remodeling could affect the expression of controls (GAPDH, 18-S rRNA respectively) for immunoblots and RT-PCR analyses; however, this proved not to be the case (supplemental Table VII).

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Disclosures

None. .

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