Cardiac Resynchronization Therapy Corrects Dyssynchrony-Induced Regional Gene Expression Changes on a Genomic Level

Andreas S. Barth, MD; Takeshi Aiba, MD, PhD; Victoria Halperin, MSc; Deborah DiSilvestre, MSc; Khalid Chakir, PhD; Carlo Colantuoni, PhD; Richard S. Tunin, MSc; Victoria Lea Dimaano, MD; Wayne Yu, PhD; Theodore P. Abraham, MD; David A. Kass, MD; Gordon F. Tomaselli, MD

Background—Cardiac electromechanical dyssynchrony causes regional disparities in workload, oxygen consumption, and myocardial perfusion within the left ventricle. We hypothesized that such dyssynchrony also induces region-specific alterations in the myocardial transcriptome that are corrected by cardiac resynchronization therapy (CRT).

Methods and Results—Adult dogs underwent left bundle branch ablation and right atrial pacing at 200 bpm for either 6 weeks (dyssynchronous heart failure, n=12) or 3 weeks, followed by 3 weeks of resynchronization by biventricular pacing at the same pacing rate (CRT, n=10). Control animals without left bundle branch block were not paced (n=13). At 6 weeks, RNA was isolated from the anterior and lateral left ventricular (LV) walls and hybridized onto canine-specific 44K microarrays. Echocardiographically, CRT led to a significant decrease in the dyssynchrony index, while dyssynchronous heart failure and CRT animals had a comparable degree of LV dysfunction. In dyssynchronous heart failure, changes in gene expression were primarily observed in the anterior LV, resulting in increased regional heterogeneity of gene expression within the LV. Dyssynchrony-induced expression changes in 1050 transcripts were reversed by CRT to levels of nonpaced hearts (false discovery rate <5%). CRT remodeled transcripts with metabolic and cell signaling function and greatly reduced regional heterogeneity of gene expression as compared with dyssynchronous heart failure.

Conclusions—Our results demonstrate a profound effect of electromechanical dyssynchrony on the regional cardiac transcriptome, causing gene expression changes primarily in the anterior LV wall. CRT corrected the alterations in gene expression in the anterior wall, supporting a global effect of biventricular pacing on the ventricular transcriptome that extends beyond the pacing site in the lateral wall. (Circ Cardiovasc Genet. 2009;2:371-378.)

Key Words: conduction ■ electrical stimulation ■ remodeling ■ cardiac resynchronization therapy ■ heart failure ■ gene expression ■ microarray

Nearly 5 million Americans suffer from heart failure (HF), and more than 250 000 die annually.1 Asymmetric contraction resulting from an intraventricular conduction delay is present in ≈30% of patients with HF2 and has been identified as an independent predictor of mortality in patients with HF.3–5 A left bundle branch block decreases regional loading, contractile work, myocardial blood flow, and oxygen consumption in the early-activated anterior myocardium, whereas these parameters are increased in the late-activated lateral LV.6,7 Biventricular stimulation or cardiac resynchronization therapy (CRT) has been developed to treat this disorder. It improves contractile synchrony, systolic function and rehomogenizes regional workload; and in patients, improves clinical symptoms and survival.8,9

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We first reported that dyssynchronous HF (DHF) also leads to regional disparities of protein expression, notably in stress-response kinases and cytokines,10,11 with enhanced levels in the higher stress (late-activated) lateral wall. More recently, we showed that CRT can rehomogenize these changes.10 However, our previous analysis was focused on individual proteins, and it most likely missed a much broader impact of dyssynchrony and CRT on regional molecular expression patterns. To test this, we used a global gene expression profiling approach in a recently developed canine model of DHF and CRT,10 examining
Table 1. Phenotypic Characterization

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>DHF</th>
<th>CRT</th>
<th>ANOVA</th>
</tr>
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<tbody>
<tr>
<td>Dyssynchrony index, td</td>
<td>30±1.2</td>
<td>68±4.6*</td>
<td>31.3±5.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EF, %</td>
<td>66.7±3.1</td>
<td>24.8±2.6†</td>
<td>33.1±2.6‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>34±2.7</td>
<td>21.5±2.4</td>
<td>31.9±3.8†</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>End-diastolic pressure, mm Hg</td>
<td>6.2±1.4</td>
<td>30.9±2.5*</td>
<td>28.8±2.5*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dP/dTmx/IP, sec&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>27.6±1.4</td>
<td>13.2±0.6†</td>
<td>16.9±1.2‡</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Echocardiographic and invasive hemodynamic measurements of DHF (n=17), CRT (n=15), and nonfailing (n=6) dogs. Unpaired t-test, p<0.05: *compared to control; ‡compared to CHF.

regional disparities in the cardiac transcriptome in DHF and determining the capacity of CRT to ameliorate these abnormalities.

Methods

CRT in a Canine Tachypacing-Induced HF Model

Details of the animal model have been described previously. Briefly, adult male mongrel dogs (n=22) underwent left bundle branch radiofrequency ablation and later received bipolar epicardial leads (Medtronic, Minneapolis, Minn) implanted on the right atrium, right ventricular free wall, and lateral left ventricle (LV). For 3 weeks, all dogs were subjected to rapid atrial pacing (200 minutes<sup>−1</sup>) to induce DHF. Dogs were subsequently divided into 2 groups for an additional pacing period of 3 weeks: while DHF animals (n=12) continued to receive atrial tachypacing, CRT dogs (n=10) received biventricular tachypacing at the same rate during the latter half of the pacing protocol. Control animals without left bundle branch block were not paced (NF, n=13). All protocols followed the USDA and NIH guidelines and were approved by our institution’s Animal Care and Use Committee.

Echocardiography and Hemodynamic Recordings

Chamber function was assessed by 2-dimensional echocardiography with tissue Doppler imaging (at the 3- and 6-week time points) and by invasive catheterization at the time of death. The details have been previously reported.

Microarray Hybridization and Statistical Analysis

Total RNA was isolated with TRIzol reagent (Invitrogen, Carlsbad, Calif) from the subendocardium of the anterior and lateral LV walls in the distribution of the left anterior descending (LAD) and left circumflex artery, respectively. Following a 1-color design in 11 NF, 10 DHF, and 9 CRT animals, RNA was labeled with Cy3 and hybridized onto Agilent 44K canine-specific microarrays. The RNA spike-ins of Agilent were mixed with the sample and cohybridized to the arrays following the manufacturer’s instructions. The quality of the microarray hybridizations was verified by controlling for the dynamic range, saturation, pixel noise, grid misalignment, and signal-to-noise ratio.

To validate the results from 1-color microarray experiments, where RNA isolated from the anterior and lateral walls from the same heart was hybridized onto 2 separate arrays, experiments were also performed using a 2-color design in a subset of animals (6 NF, 5 DHF, and 5 CRT dogs), partially overlapping those used for the 1-color design. In these experiments, corresponding anterior and lateral samples from the same LV were labeled with Cy3 and Cy5 (including dye swap experiments) and hybridized onto the same array to achieve a direct comparison of the relative gene expression.

![Figure 1. KEGG pathway analysis in tachycardia pacing-induced heart failure. Transcripts upregulated and downregulated by ventricular tachypacing in canine ventricular myocardium are represented by white and black columns, respectively, and shown as percentage of 11 major KEGG pathways (studies a–c). Study a shows the comparison of anterior samples between NF and DHF hearts in the current Agilent-based microarray study. Studies b and c show the results of 2 publicly available Affymetrix microarray datasets (Gene Expression Omnibus accession numbers 9794 and 5247, respectively) comparing myocardial tissue derived from the anterior LV wall from nonpaced animals with dogs that were tachypaced for at least 3 weeks. Normalized data were downloaded from Gene Expression Omnibus and analyzed using the same methods used in this study (a). For all KEGG pathways shown, a probability value of <0.05 (Fisher exact test implemented in the “FatiGO+” tool) was achieved in at least 2 of the 3 different canine tachypacing studies presented. It is evident that energy-deriving processes, including oxidative phosphorylation and tricarboxylic acid cycle, are greatly downregulated in pacing-induced HF, whereas various cell signaling pathways and extracellular matrix components are upregulated. The high concordance of disease-specific gene expression patterns across independent studies and different microarray platforms serves as an independent validation of our results and suggests a common disease-specific genomic fingerprint.](http://circgenetics.ahajournals.org/DownloadedFrom)
in different regions of the same heart. Additional validation included comparison of the data from this study to 2 publicly available datasets of DHF in dogs with right ventricular pacing-induced LV dysfunction (Gene Expression Omnibus accession numbers GSE5247 and GSE9794). Preprocessing and most of the statistical analysis were done using R (www.r-project.org) and Bioconductor (www.bioconductor.org). Microarray data were normalized using quantile normalization implemented in Bioconductor’s “affy”-package (for 1-color data) or loess normalization implemented in Bioconductor’s “limma”-package (for 2-color microarray data). Complying with minimum information about a microarray experiment standards (MIAME), microarray data have been submitted to a public repository (Gene Expression Omnibus; the SuperSeries accession number GSE14661 includes GSE14327 [1-color design data] and GSE14338 [2-color design data]).

To determine differentially expressed genes, multiclass and unpaired 2-class significance analysis of microarrays (SAM) was used. Differences in gene expression were regarded as statistically significant if a false discovery rate (FDR) of \( q < 0.05 \) was achieved. Functional annotation of differentially expressed genes was based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways database. Overrepresentation of specific KEGG pathways in a gene set was statistically analyzed by “FatiGO” and the Database for Annotation, Visualization, and Integrated Discovery.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

DHF and Biventricular Pacing

The hemodynamic features of the canine DHF and CRT model have been reported previously, and results obtained more recently from a larger cohort are summarized in Table 1. Compared with normal controls, DHF ventricles display marked dyssynchrony that was restored to normal levels with CRT. Significant differences were observed in ejection fraction (increase of \( \sim 10\% \)), stroke volume, and LV contractility assessed by \( \frac{dP}{dT_{\text{max}}} \) normalized to instantaneous developed...
Regional Regulation of Gene Expression in DHF

Figure 1 shows the relative distribution of significantly upregulated and downregulated transcripts for 11 KEGG pathways in the anterior myocardium, comparing nonfailing and paced (DHF) animals. Energy-deriving processes, including oxidative phosphorylation, tricarboxylic acid cycle, fatty acid and amino acid metabolism, were concomitantly downregulated in failing myocardium, whereas various cell signaling pathways and extracellular matrix components were upregulated. To validate the reproducibility of the results obtained with the Agilent microarray platform, we compared our results with 2 publicly available datasets of pacing-induced (single site and dyssynchronous) HF in dogs, generated with Affymetrix microarrays with tissue from the anterior LV wall (FDR \(< 5\%\) for all studies).\(^{15,16}\) The high concordance of gene expression patterns among these 3 studies underscores the reproducibility of our findings and highlights the robust genomic fingerprint of DHF in the anterior myocardium.

Dyssynchronous contraction of the LV associated with left bundle branch block imposes greater stress on the lateral wall compared with the anterior LV. To test the hypothesis that DHF alters gene expression patterns in a region-specific fashion, samples from anterior and lateral LV myocardium were examined separately in nonfailing and DHF animals.

Separate analysis of the regional transcriptome from the same hearts (NF, \(n = 11\); DHF, \(n = 10\)), identified \(> 6\) times as many genes that were differentially expressed between NF and DHF hearts in anterior compared with lateral LV myocardium (2173 versus 346 transcripts, respectively; SAM with an FDR \(< 5\%\); Table 2 and supplemental Figure I).\(^{15,16}\) Besides these quantitative differences, important qualitative differences were evident for DHF-induced gene expression changes in the anterior and lateral myocardium. While downregulation of energy-deriving processes, including oxidative phosphorylation and tricarboxylic acid cycle, was more pronounced in the anterior compared with the lateral wall, regulation of cell signaling pathways and extracellular matrix components displayed distinct patterns in the lateral and anterior LV myocardium in dyssynchronous HF. For instance, cell signaling pathways were upregulated in the anterior LV wall but predominantly downregulated in the lateral LV wall (Figure 2, upper panel). The small number of regulated genes found in the lateral myocardium of DHF hearts limited the statistical power of the KEGG pathway analysis, thus an additional analysis was performed where KEGG pathways of the first 1000 upregulated and downregulated transcripts were compared in both regions, irrespective of the significance level (Figure 2, lower panel). This approach was used to determine whether the changes in gene expression in the lateral wall differ only quantitatively from those in the anterior LV wall. The metabolic gene classes were still downregulated; however, the pattern of gene expression in cell signaling pathways and extracellular matrix components differed considerably from the robust genomic fingerprint observed in anterior myocardium in DHF (Figure 1), suggesting a quantitatively and qualitatively different transcriptomic response to electromechanical dyssynchrony in the anterior and lateral LV.

Biventricular Pacing Reverses Gene Expression Changes

Separate analysis of gene expression in anterior and lateral LV samples identified only 7 differentially expressed transcripts between DHF and CRT hearts (Table 2 and supplemental Table I). However, when anterior and lateral samples from the same hearts were paired by examining the difference in gene expression between the 2 regions (anterior minus lateral LV wall), a large number of transcripts were found to be differentially expressed between DHF and CRT hearts (Table 2). An unsupervised clustering of 1050 transcripts, identified by SAM multiclass analysis of regional differences in gene expression between anterior and lateral wall for NF, DHF, and CRT hearts, revealed that CRT hearts clustered with NF, rather than with gene expression patterns from DHF samples (Figure 3A; the gene list is provided in supplemental Table II). An identical picture emerged when data from the 2-color microarray design were clustered (Figure 3B). The latter experiments also revealed few gene expression changes between the anterior and lateral regions in the NF myocardium (Figure 4). This was substantiated by SAM, with only 2 transcripts being differentially expressed between anterior...
and lateral regions in nonfailing myocardium (LIM domain only protein 3 in anterior myocardium and glutathione S-transferase P in lateral myocardium, FDR 5% in 2-color array data). In contrast, there was a marked heterogeneity in gene expression between anterior and lateral LV regions in DHF that was reduced by CRT to levels comparable with NF hearts (Figure 4). Moreover, CRT partially reversed DHF-induced gene expression changes (Figure 5), as evidenced by KEGG pathway analysis; transcripts of metabolic activity were upregulated in the anterior wall, whereas transcripts encoding for cell signaling pathways and extracellular matrix components were downregulated. Therefore, CRT restored the relative balance of gene expression between the anterior and lateral LV, eg, expression of mitogen-activated protein kinase pathway signaling and extracellular matrix components was reduced in the anterior wall but increased in lateral LV (Figure 5).

**Discussion**

By using an unbiased and global assessment of transcriptional activity in a large animal model of DHF, we found that dyssynchrony-induced changes in gene expression were more pronounced in the anterior compared with the lateral LV. The genes that showed significant heterogeneity in regional expression with dyssynchrony are involved in important processes such as metabolic pathways, extracellular matrix remodeling, and myocardial stress responses. The disparity in the number of regulated transcripts between the early- and late-activated LV regions gave rise to an increased regional heterogeneity of gene expression within the dyssynchronously contracting myocardium. Remarkably, dyssynchrony-induced expression changes were reversed by CRT to levels in NF hearts, as evident by a reduced regional heterogeneity of gene expression and prominent reverse remodeling of transcripts with metabolic and cell signaling function.

A number of factors have been shown to regulate transcriptional activity in the heart, including contractile activity, stretch, myocardial perfusion, and metabolism.21–23 Because all of these parameters are altered in a region-specific fashion in DHF, they could account for the differential transcriptional response of the anterior and lateral walls. It is well known that cardiac dyssynchrony, whether caused by a left bundle branch block or right ventricular free wall pacing, decreases regional loading, contractile work, myocardial blood flow, and oxygen consumption in the early-activated anterior myocardium. For instance, the regional pressure-strain loop area, which corresponds to the external work performed, is reduced to a greater extent in the anterior compared with the lateral wall in DHF hearts.13 In line with this finding, downregulation of metabolic transcripts was significantly greater in anterior compared with lateral LV regions. Biventricular pacing improves contractile timing, thereby increasing regional work in the anterior wall while reducing work in the lateral LV region. Experimentally, this has been shown to couple with rebalanc-
ing of glucose metabolism and myocardial blood flow (rising in the anterior and declining in lateral walls), and such findings are consistent with CRT-associated increases in transcripts levels encoding oxidative phosphorylation and various metabolic pathways in anterior samples observed in this study. In another study performed in this model, we have observed an upregulation of proteins in various metabolic pathways in CRT by examining the myocardial mitochondrial proteome. Indeed, nearly 50% of the protein changes involved subunits of the electron transport chain, the majority of which displayed marked upregulation with CRT. Additionally, key enzymes in anaplerotic pathways, such as branched chain amino acid oxidation and pyruvate carboxylation, were increased, suggesting that CRT may increase the pool of Krebs cycle intermediates to fuel oxidative phosphorylation.

Although CRT effectively restored dyssynchrony-induced gene expression changes in this model, it did not correct overall HF-induced transcriptomic alterations. The changes in gene expression levels brought about by CRT were only significant when a paired design for anterior and lateral LV region of each heart was used, ie, influencing heterogeneity of expression, but far less when anterior and lateral regions in DHF and CRT hearts were compared separately (Table 2). In our view, 2 main findings suggest that even this small yet significant effect of CRT on the cardiac transcriptome likely has global effects on heart function. First, dyssynchrony-induced expression changes tended to aggravate the HF-related transcriptomic signature (Figures 2 and 5). In DHF, for example, various cell signaling and extracellular matrix remodeling pathways were upregulated in the early-activated anterior but downregulated in the late-activated lateral LV wall. In contrast, biventricular pacing partially reversed cell signaling and extracellular matrix remodeling changes by restoring the relative balance in gene expression levels between anterior and lateral wall (downregulation in anterior regions with concomitant upregulation of mRNA levels of these pathways in the lateral wall). Thus, dyssynchrony-induced expression changes could contribute to the decline in EF in DHF animals between 3 and 6 weeks of pacing, whereas CRT animals showed a modest increase in EF during the same period (Table 1). Second, dyssynchrony-induced gene expression changes significantly increased the heterogeneity of gene expression within early- and late-activated LV wall regions. It is tempting to speculate that this increased heterogeneity within the LV wall also reflects regionally heterogeneous remodeling of ion channels that exist in this HF model and possibly increased QT dispersion. In a study that examined QT interval duration in relation to CRT, Berger et al found that QT dispersion increased during right ventricular and decreased during biventricular pacing, thus paralleling changes observed for action potential duration with DHF and CRT in isolated cardiomyocytes.

In summary, we demonstrate a profound effect of electric activation on the regional cardiac transcriptome and provide unique insights into transcriptome-wide molecular processes underlying transcriptomic remodeling in CRT. The dyssynchronous failing heart is not simply worse HF but a form of disease with profound regional gene expression disparities.
Moreover, we show for the first time that by recoordinating contraction, such expression heterogeneity can be essentially returned to normal, even in a failing heart, on a genome-wide level. This may point to a more biological method to assess the impact of CRT.

**Study Limitations**

Pacing-induced HF is a widely used animal model of nonischemic HF that shares major electrophysiological (action potential prolongation, high incidence of sudden cardiac death, atrial arrhythmias), morphological (biventricular dilation), and functional (depressed contractility) hallmarks of human HF. However, tachypacing-induced HF does not mimic all features of human HF because the changes in myocardial structure occurring with tachypacing are dissimilar to clinical forms of HF caused by chronic ischemia or hypertensive disease. Thus, extrapolation of the findings from this HF model to clinical forms of HF should be done with caution because this model dose not fully represent the complex clinical spectrum of HF. Additionally, pharmacological interventions known to disrupt the neurohormonal dysfunction in HF (eg, β-blockade or angiotensin-converting enzyme inhibition) were not used in this model. Because this study was designed to examine the effects of CRT with ongoing HF, tachypacing was maintained throughout the study. Thus, if anything, this model might be predicted to delay benefits that might come from resynchronization itself, although improvement was still clearly documented. Given the higher heart rate necessary to induce and maintain HF in this model, the processes of remodeling and reverse remodeling may differ from the clinical HF syndrome. Additional studies will be needed to differentiate transcriptional changes associated with altered electric activation of the ventricles and dysynchronous mechanical contraction independent of HF.

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**Disclosures**

None.

**References**


**CLINICAL PERSPECTIVE**

Congestive heart failure is a leading cause of morbidity and mortality worldwide. Over the past decade, one of the most significant therapeutic advances in heart failure treatment has been biventricular pacing (cardiac resynchronization therapy [CRT]). CRT can both acutely and chronically increase systolic function, improve the efficiency of contraction, and prolong survival in patients with left-sided intraventricular conduction delay. However, the mechanisms underlying the benefit of CRT remain elusive. By using microarray-based studies of gene expression, we provide for the first time detailed and comprehensive insights into the transcriptional processes associated with dyssynchronous electromechanical activation. These experiments were conducted in a well-controlled large animal model, enabling tissue sampling from early-activated anterior and late-activated lateral left ventricular regions in dyssynchronous heart failure. As a result, we report a profound effect of electromechanical dyssynchrony on the regional cardiac transcriptome. Moreover, we show that, by recoordinating contraction, the heterogeneity of gene expression can be greatly reduced, even in a failing heart, on a genome-wide level. This may point to a more biological method to assess the impact of CRT. A better understanding of the molecular processes associated with the reverse remodeling in CRT will help to optimize and refine patient selection, device settings, and outcome assessment.
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SUPPLEMENTAL MATERIAL

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Online Figure 1. Clustering of regional differences between anterior and lateral myocardium. Unsupervised clustering of differentially expressed transcripts identified by SAM (NF vs. DHF, false discovery rate <5%) using Euclidean distance for one-color microarray data, separately for anterior and lateral myocardium (panel A and B, respectively). Each row represents data for one gene. The gene expression level is color-coded with yellow and blue representing low and high expression, respectively. In pacing-induced HF, transcriptional changes were more pronounced in the anterior wall, with more than 6-times more transcripts showing statistically significant changes compared to the lateral wall.
**Online Table 1.** Differentially expressed transcripts between CRT and DHF hearts in anterior and lateral myocardium (Significance Analysis of Microarrays, SAM, two class, unpaired, false discovery rate <5%).

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<th>Anterior</th>
<th>Agilent-ID</th>
<th>Gene Symbol</th>
<th>Fold Change (CRT vs. DHF)</th>
<th>q-value(%)</th>
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<td>ANKRD23</td>
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<td>APP</td>
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<td>0.63</td>
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<table>
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<th>Lateral</th>
<th>Agilent-ID</th>
<th>Gene Symbol</th>
<th>Fold Change (CRT vs. DHF)</th>
<th>q-value(%)</th>
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<td>PRKAR1A</td>
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<td>A_11_P000005798</td>
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<td>RTN4IP1</td>
<td>0.78</td>
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Online Table 2. Regional expression changes (anterior - lateral myocardium) in NF, DHF and CRT hearts (Significance Analysis of Microarrays, SAM, multiclass, false discovery rate <5%).

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<tr>
<th>Agilent ID</th>
<th>Gene</th>
<th>contrast (NF, ANT-LAT)</th>
<th>contrast (DHF, ANT-LAT)</th>
<th>contrast (CRT, ANT-LAT)</th>
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