Differential Gene Expression and Genomic Patient Stratification Following Left Ventricular Assist Device Support

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OBJECTIVES
We sought to determine whether mechanical unloading of the failing human heart with a left ventricular assist device (LVAD) results in significant changes in overall left ventricular gene expression.

BACKGROUND
Mechanical circulatory support by LVAD in end-stage human heart failure (HF) can result in beneficial reverse remodeling of myocardial structure and function. The molecular mechanisms behind this salutary process are not well understood.

METHODS
Left ventricular samples from six male patients were harvested during LVAD placement and subsequently at the time of explantation. Cardiac gene expression was determined using oligonucleotide microarrays.

RESULTS
Paired t test analysis revealed numerous genes that were regulated in a statistically significant fashion, including the downregulation of several previously studied genes. Further statistical analysis revealed that the overall gene expression profiles could significantly distinguish pre- and post-LVAD status. Interestingly, the data also identified two distinct groups among the pre-LVAD failing hearts, in which there was blind segregation of patients based on HF etiology. In addition to the substantial divergence in genomic profiles for these two HF groups, there were significant differences in their corresponding LVAD-mediated regulation of gene expression.

CONCLUSIONS
Support with an LVAD in HF induces significant changes in myocardial gene expression, as pre- and post-LVAD hearts demonstrate significantly distinct genomic footprints. Thus, reverse remodeling is associated with a specific pattern of gene expression. Moreover, we found that deoxyribonucleic acid microarray technology could distinguish, in a blind manner, patients with different HF etiologies. Expansion of this study and further development of these statistical methods may facilitate prognostic prediction of the individual patient response to LVAD support. (J Am Coll Cardiol 2003;41:1096–106) © 2003 by the American College of Cardiology Foundation

Although heart transplantation is an effective interventional therapy for end-stage heart failure (HF), donor hearts are consistently in limited supply. Hence, patients who experience clinical deterioration before transplant availability are frequently given mechanical circulatory support in the form of a left ventricular assist device (LVAD), employed as a "bridge to transplant." To date, the utility, efficacy, and safety of LVAD support as a bridge to transplant has been widely recognized (1,2). Importantly, LVAD support, similar to all drugs that exert beneficial effects on mortality in patients with HF, also favorably impacts the left ventricular (LV) remodeling process (3–6). Recent evidence has demonstrated that unloading the LV in patients with end-stage HF by LVAD support can lead toward reverse remodeling of myocardial structure and function (6–15). Studies of LVAD-mediated reverse remodeling have described changes such as decreased myocyte size (8,12,14), increased myocyte contractility (8,13), improved mitochondrial function (15), decreased myocyte apoptosis, and normalized mitogen-activated protein kinase signaling (12). However, the overall molecular mechanisms of the reverse remodeling process are not yet well understood. Owing to the beneficial effects of LVAD support, it is likely that identification of the mechanisms responsible for the associated reverse remodeling will lead to potentially novel diagnostic and therapeutic targets for the treatment of HF.

Recent investigations following LVAD support have focused on the expression of individual genes or their protein products and have found a significant regulation of several, including downregulation of natriuretic peptides (16,17), collagen (18,19), dystrophin (20,21), interleukins (22), matrix metalloproteinases (23), metallothionein (24), and tumor necrosis factor-α (25). These results demonstrate that mechanical support of the failing myocardium can indeed induce significant changes in gene and/or protein expression. Such reports have given us initial insight into some of the molecular mechanisms that may be involved in LVAD-mediated myocardial recovery. However, the recent development of oligonucleotide microarray technology can simultaneously examine the expression of thousands of genes in a single sample, thereby allowing for a more comprehensive understanding of the molecular events underlying LVAD-mediated reverse remodeling.
genes. This technology could provide important insight into a substantially greater number of specific genes, as well as overall gene expression profiles characteristic of the reverse remodeling process.

Rather than target a limited number of genes of interest, we wished to gain broader insight into the potential molecular mechanisms associated with LVAD-associated reverse remodeling, particularly with regard to statistically significant changes in myocardial gene expression. Herein we describe experiments designed to test overall gene expression from patients both before and after LVAD support using oligonucleotide microarray technology coupled with various statistical analyses to: 1) validate that our approach using microarrays recapitulates previously reported changes in gene expression; 2) report statistically relevant changes in gene expression detectable by these methods; 3) determine whether differential patterns of overall gene expression can indeed distinguish between patients before and after LVAD support; and 4) investigate the possibility of using differential gene expression to more accurately stratify patients both before and after LVAD support, in concordance with other clinical data.

METHODS

Tissue sampling and ribonucleic acid (RNA) extraction. Tissue was obtained from the LV free wall (toward the apex) of six male patients at the time of LVAD placement (HeartMate, Thoratec Corp., Pleasanton, California) and at the time of myocardial explantation. The duration of LVAD support in all patients ranged from two to four months. The LV tissue obtained from surgery was immediately frozen in liquid nitrogen and stored at $-80^\circ$C. All surgical procedures and tissue harvesting were performed in concordance with the National Institutes of Health and Duke University Institutional Review Board guidelines. Caution was exercised to utilize only viable, nonischemic cardiac tissue from both pre- and post-LVAD samples for RNA extraction. Total RNA was extracted using the Qiagen RNeasy kit (Qiagen, Inc., Valencia, California), including the proteinase K digestion recommended by the manufacturer for RNA extraction from muscular tissue. Total RNA quality was assessed by 1.2% agarose-formaldehyde gel electrophoresis to visualize the 18S and 28S ribosomal ribonucleic acid (rRNA). Subsequently, RNA quality was more rigorously determined using Agilent RNA 6000 chips coupled with the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, California), where 500 ng was used to determine the 18S:28S rRNA ratio. All RNA used in the current study met the minimum requirement of at least a 1.8 ratio of 18S:28S rRNA. The RNA aliquots were stored at $-80^\circ$C before use.

Microarray analysis. Expression analysis was performed using Affymetrix HuGeneFL GeneChips, which allow detection of approximately 6,800 known genes or novel clones with homology to known genes (Affymetrix, Santa Clara, California). Target chromosomal RNA was prepared according to the manufacturer’s protocol. Arrays were hybridized with the targets at $45^\circ$C for 16 h, then washed and stained using the GeneChip Fluidics Station. The deoxyribonucleic acid (DNA) chips were scanned with the Gene Chip scanner, and the scanning results were processed by the GeneChip Expression Analysis Algorithm (version 3.2), with a target intensity of 500 (Affymetrix). Affymetrix chip processing was performed at the Duke University Genome Core Facility. In a concerted effort to minimize inherent variability, all chips were processed on the same day, using the same manufacturing lot of reagents and same Affymetrix scanner, and all had scaling factors within 10% of the group mean value.

Statistical analysis. Raw average difference values provided by the Affymetrix analysis from each chip were used for all statistical analyses. Paired $t$ tests and multidimensional scaling (MDS) of total gene expression profiles were performed using Partek software (Partek, Inc., St. Charles, Missouri). In the simplest of terms, MDS is mapping of high-dimensional data (~6,800 genes per patient) into low-dimensional space, such that the distances between points mapped in low-dimensional Euclidean space represent the similarities, dissimilarities, or distances of the original data. In this case, the distance between points in the images approximates a dissimilarity between points in high-dimensional space. Herein, metric MDS was performed based on the Pearson dissimilarity (defined as $[1 - r]/2$, where $r$ is the Pearson correlation) and mapped into two-dimensional space (initialized with points drawn randomly from a uniform distribution in the range of $-1$ to 1) (26). Figures are generated by MDS to minimize a measure of badness of fit, called stress, which depends on the point-to-point dissimilarities in the data compared with the distances in low-dimensional space. All images presented herein had stress values from 0.002 to 0.031. In these images, axes $x$ and $y$ represent the two Euclidean dimensions into which the Pearson dissimilarities have been mapped. Hierarchical clustering of patients according to total or statistically filtered gene expression was performed using Cluster and TreeView, made available from Michael Eisen’s laboratory, using default settings, with hierarchical clustering based on uncentered correlations with average linkage clustering. Clustering of data according to biologic function was performed using the coded electronic life library suite of software from Incellico, Inc. (Durham,
Table 1. Validation of Microarray Method

<table>
<thead>
<tr>
<th>GenBank No.</th>
<th>Gene</th>
<th>p Value</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>M31776</td>
<td>Brain natriuretic peptide</td>
<td>0.046</td>
<td>-4.2</td>
</tr>
<tr>
<td>X60382</td>
<td>Collagen</td>
<td>0.047</td>
<td>-3.0</td>
</tr>
<tr>
<td>L35854</td>
<td>Dystrophin</td>
<td>0.019</td>
<td>-5.6</td>
</tr>
<tr>
<td>U11877</td>
<td>Interleukin-8</td>
<td>0.010</td>
<td>-9.3</td>
</tr>
<tr>
<td>X64177</td>
<td>Metallothionein</td>
<td>0.024</td>
<td>-2.4</td>
</tr>
<tr>
<td>D50477</td>
<td>Matrix metalloproteinase</td>
<td>0.036</td>
<td>-4.0</td>
</tr>
<tr>
<td>U12595</td>
<td>Tumor necrosis factor-α</td>
<td>0.032</td>
<td>-1.6</td>
</tr>
</tbody>
</table>

Statistically significant regulation of genes detected by our methods for which similar changes have previously been reported following left ventricular assist device support (11–20). Statistical significance (p < 0.05) determined by the paired t test.

North Carolina). For all tests, where applicable, p < 0.05 was considered significant. It should be noted that for a paired t test, there are caveats regarding multiple comparisons when analyzing ~6,800 genes across only 12 samples. The current data set does not withstand correction for multiple comparisons; thus, we do not place a high degree of confidence in the individual, gene-specific results from the paired t test, particularly with regard to the smaller paired groups of three patients, with the exception of those genes we further validated using Northern blot analysis. Fold change calculations were performed using methods previously published (27). Briefly, the mean value of each group was taken, and a mean average difference of <0.20 was brought up to 20, based on previous data suggesting that average difference values below 20 were unreliable or undetectable. The mean average difference of each group was then compared, and the relative fold change was calculated based on the comparison.

RESULTS

LVAD-mediated regulation of gene expression. To elucidate genomic changes that occur following ventricular unloading in the LVAD-associated reverse remodeling process, we analyzed differential gene expression in paired LV free wall samples from six male patients with end-stage cardiomyopathy obtained at the time of device placement (pre-LVAD) and following ~2 months of mechanical support (post-LVAD) (six paired samples). Gene expression values were determined using HuGene FL oligonucleotide microarrays from Affymetrix, which provide detection of ~6,800 known human genes (or novel clones with homology to know human genes). Paired statistical analysis of the gene expression data revealed a significant reduction in expression of genes previously reported to be downregulated following LVAD support (at the level of messenger RNA and/or protein expression), including collagen (18,19), dystrophin (20,21), interleukin-8 (22), matrix metalloproteinase (23), metallothionein (24), and tumor necrosis factor-α (p < 0.05) (Table 1) (25). Furthermore, several hallmark genes associated with HF, such as brain natriuretic peptide (BNP), were also significantly downregulated following LVAD support, as previously described (p < 0.05) (Table 1) (16,17). In all, 530 genes were regulated in a statistically significant manner in a comparison of pre- and post-LVAD samples: 295 genes were upregulated and 235 were downregulated (supplemental Data of Table 1). Among these were several genes of particular interest in the setting of HF; however, numerous others have not been previously associated with cardiac function, HF, or reverse remodeling.

Statistically distinct pre- and post-LVAD gene expression profiles. Although investigation of statistically significant differential gene expression at the individual gene level is important, we also wanted to determine whether the overall genomic profile could segregate patients according to pre- or post-LVAD status. Therefore, we wished to visualize and analyze overall data structure in dimensional space using multidimensional scaling (see Methods). This analysis demonstrated a distinct separation between the pre- and post-LVAD groups, indicating that there is indeed a genomic signature of changes in gene expression associated with LV reverse remodeling following LVAD support (Fig. 1). To further analyze the effect of LVAD support on gene expression, Figure 1 also presents the Pearson correlation matrix on which the MDS was based. The mean correlation was 0.884 within the pre-LVAD group and 0.927 within the post-LVAD group, but only 0.802 between the groups. Of the 462 possible assignments of the 12 profiles to two groups of six patients, the assignment with the lowest mean correlation between groups was indeed that of the pre- and post-LVAD groups (p = 0.002), clearly demonstrating the robust and significant effect of LVAD support on gene expression. Interestingly, both methods suggest there was greater gene expression diversity in the pre-LVAD group, including a separation of the pre-LVAD group into two groups of three patients, although there was a greater similarity in the entire post-LVAD group gene expression profile.

Gene expression profile segregates patients concordant with HF etiology. Subsequent analysis of individual patient data (to which the investigators were previously blinded) showed that members of both groups shared several characteristics that could not adequately distinguish them, including age, duration of diagnosed illness, duration of LVAD support, and similar pharmacologic treatment before LVAD support (Table 2). However, there was a clear segregation of these two groups of patients based on their apparent HF etiology, as determined both clinically and pathologically after explantation (Table 2). The pre-LVAD group of three patients with gene expression patterns most distinct from the post-LVAD group had nonischemic, idiopathic dilated cardiomyopathy (Table 2 [nonischemic patients 1-3], Fig. 2A), whereas the pre-LVAD patients most similar to the post-LVAD group had ischemic cardiomyopathy (Table 2 [ischemic patients 4-6], Fig. 2A). In light of the fact that MDS of the entire gene expression data revealed patient distinction according to HF etiology, we wished to determine whether data from the genes found by the paired t test to be significantly regulated...
following LVAD support would demonstrate a similar pattern of distinction. Importantly, gene expression data from the 530 genes found to be significantly altered after LVAD support (by paired $t$ test), analyzed by MDS, showed a similar pattern of separation between the pre- and post-LVAD groups, as well as a more distinct segregation of the pre-LVAD group when preclassified according to HF etiology (Fig. 2B). Separation of the pre- and post-LVAD groups, similar to that described earlier using MDS, was found by the conventional Cluster and TreeView programs (another method of statistically valid visualization and analysis of data structure), accompanied by separation of pre-LVAD patients according to their clinical history and HF etiology (Fig. 3). Thus, the data clearly demonstrate that individual LVAD status and HF etiology could be distinguished using multiple statistical methods to analyze transcriptional profiling. Due to the lack of a current “gold standard” method for analyzing gene expression data sets, we employed several statistical methods and believe it is an important finding that all methods reached similar conclusions.

**HF etiology-specific changes in gene expression.** Our ability to classify patients according to HF etiology by gene expression profile warranted further investigation of
<table>
<thead>
<tr>
<th>Pt. No.</th>
<th>Clinical Description</th>
<th>Pathologic Description</th>
<th>Diagnosis*</th>
<th>Age (yrs)</th>
<th>Gender</th>
<th>LVAD Duration (days)</th>
<th>LVEF</th>
<th>Patient History</th>
<th>Medications†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nonischemic IDC, CHF, and TdeP (Mod. enl.)</td>
<td>IDC, very mild CAD, mild fibrosis (nonischemic distribution)</td>
<td>5</td>
<td>61</td>
<td>M</td>
<td>47</td>
<td>15%</td>
<td>Hypercholesterolemia, lipidemia, gout</td>
<td>Aspirin, Ativan, Coumadin, Lanoxin, Lasix, Toprol, Vasotec, Zocor, Zyrtec</td>
</tr>
<tr>
<td>2</td>
<td>Nonischemic IDC, CHF (HB) (Sev. enl.)</td>
<td>IDC, no CAD, mild fibrosis immediately surrounding LVAD tube</td>
<td>5</td>
<td>52</td>
<td>M</td>
<td>66</td>
<td>10%</td>
<td>VT, HB, pacemaker, hyperthyroidism, PPH, acute renal failure</td>
<td>Albuterol, amiodorone, aspirin, Carvedilol, digoxin, folate, KCl, Lasix, Lisinopril, thiamine, Trizodone, Zaroxyln</td>
</tr>
<tr>
<td>3</td>
<td>Nonischemic IDC (Mod. enl.)</td>
<td>IDC, no CAD, mild fibrosis, (nonischemic distribution)</td>
<td>3</td>
<td>64</td>
<td>M</td>
<td>64</td>
<td>15%</td>
<td>Diabetes, edema, cough/sputum</td>
<td>Coumadin, digoxin, insulin, KCl, Lasix, Restoril, spironolactone, Zaroxyln</td>
</tr>
<tr>
<td>4</td>
<td>Ischemic CM, CHF, post-CABG (Mild. enl.)</td>
<td>Ischemic, CAD, bypass grafts, mild biventricular dilation</td>
<td>7</td>
<td>45</td>
<td>M</td>
<td>76</td>
<td>10%</td>
<td>Diabetes, hypertension hyperlipidemia, renal insuficiency</td>
<td>Aspirin, captopril, digoxin, insulin, Lasix, Zaroxyln</td>
</tr>
<tr>
<td>5</td>
<td>Ischemic CM, hypertensive ICM (Mild. enl.)</td>
<td>Ischemic, CAD, atherosclerosis, fibrosis, mild biventricular dilation</td>
<td>6</td>
<td>53</td>
<td>M</td>
<td>53</td>
<td>15%</td>
<td>Hypertension, AF, renal insuficiency</td>
<td>Atorvastatin, digoxin, furosemide, KCl, Losartan, spironolactone, warfarin, Zaroxyln</td>
</tr>
<tr>
<td>6</td>
<td>Ischemic CM, CAD, MI ×4, CHF (Mild. enl.)</td>
<td>Ischemic, CAD, bypass grafts, stents, multiple infarcts</td>
<td>5</td>
<td>53</td>
<td>M</td>
<td>34</td>
<td>10%</td>
<td>Diabetes, bronchitis, COPD, hypertension, hyperlipidemia</td>
<td>Accupril, aspirin, Benadryl, Colace, Combivent, digoxin, Lasix, metformin, Zantac</td>
</tr>
</tbody>
</table>

*Years since initial diagnosis of heart failure. †Medications taken prior to LVAD placement. Clinical data from patients were obtained following initial gene expression data analysis based on segregation of patients before LVAD.

AF = atrial fibrillation; CABG = coronary artery bypass grafting; CAD = coronary artery disease; CHF = congestive heart failure; CM = cardiomyopathy; COPD = chronic obstructive pulmonary disorder; HB = heart block; ICM = hypertensive ischemic cardiomyopathy; IDC = idiopathic dilated cardiomyopathy; LVEF = left ventricular ejection fraction; MI = myocardial infarction; Mod. enl. or Sev. enl. = moderately or severely dilated left ventricular chamber; PPH = primary pulmonary hypertension; Pt. = patient; TdeP = torsades de pointes; VT = ventricular tachycardia.
Figure 2. Multidimensional scaling (MDS) of pre- and post-left ventricular assist device (LVAD) gene expression. (A) Raw data classified according to segregating patient clinical characteristics were subjected to MDS. Pre-1 (yellow) and post-1 (red) represent nonischemic patients, whereas pre-2 (blue) and post-2 (green) represent ischemic patients. (B) Raw gene expression data from the 530 genes determined to be significant by paired $t$ test analysis, classified according to segregating patient clinical characteristics and subjected to MDS. The images represent convergence of the MDS algorithm, stress for both (measure of image validity) <0.05. Note the limitation of program-assigned color schemes.
LVAD-mediated changes in gene expression of ischemic and nonischemic patients. Indeed, paired $t$ test analysis demonstrated HF etiology-specific differences in gene expression following LVAD support: ischemic patients demonstrated significant changes of 247 genes (122 upregulated and 125 downregulated) (supplemental data of Table 2), whereas nonischemic patients showed a significant regulation of 784 genes (490 upregulated and 294 downregulated) (supplemental data of Table 3). These data further support our finding that the transcriptional profile of ischemic

![Figure 3](image)

Figure 3. Hierarchical clustering using Cluster and Treeview. Data for these programs require different names for each sample, hence 1-3 represent nonischemic patients and 4-6 represent ischemic patients. (A) Hierarchical clustering of raw data; note the segregation of the nonischemic patients and relative similarity of ischemic patients post-left ventricular assist device (LVAD). (B) Hierarchical clustering of raw data from the 530 genes found to be significantly regulated by the paired $t$ test. Note the distinct separation of pre- and post-LVAD, along with segregation of the nonischemic and ischemic patients pre-LVAD.

Table 3. Genes Common to Ischemic and Nonischemic Patients Before and After Left Ventricular Assist Device Support

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Description</th>
<th>Nonischemic Patients (Fold Change)</th>
<th>Ischemic Patients (Fold Change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S75578</td>
<td>4-Aminobutyrate aminotransferase</td>
<td>18.5</td>
<td>-1.3</td>
</tr>
<tr>
<td>U36221</td>
<td>Human pancreatic zymogen granule membrane protein GP-2 (a.k.a., EGF-1)</td>
<td>18.0</td>
<td>-4.5</td>
</tr>
<tr>
<td>M37755</td>
<td>Human pregnancy-specific $\beta_{-}$-glycoprotein gene PSGGA (a.k.a., actinin-1)</td>
<td>12.9</td>
<td>1.5</td>
</tr>
<tr>
<td>U07919</td>
<td>Human aldehyde dehydrogenase-6</td>
<td>12.4</td>
<td>2.5</td>
</tr>
<tr>
<td>X51956</td>
<td>Human ENO2 gene for neuron-specific (gamma) enolase</td>
<td>10.1</td>
<td>-2.5</td>
</tr>
<tr>
<td>X34789</td>
<td>H. sapiens $\alpha$-A crystallin (a.k.a., HSP-20)</td>
<td>9.9</td>
<td>-9.3</td>
</tr>
<tr>
<td>M64936</td>
<td>Human aldehyde dehydrogenase-6</td>
<td>12.4</td>
<td>2.5</td>
</tr>
<tr>
<td>U36221</td>
<td>Human pancreatic zymogen granule membrane protein GP-2 (a.k.a., EGF-1)</td>
<td>18.0</td>
<td>-4.5</td>
</tr>
<tr>
<td>M37755</td>
<td>Human pregnancy-specific $\beta_{-}$-glycoprotein gene PSGGA (a.k.a., actinin-1)</td>
<td>12.9</td>
<td>1.5</td>
</tr>
<tr>
<td>U07919</td>
<td>Human aldehyde dehydrogenase-6</td>
<td>12.4</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Paired $t$ test analyses of patients from ischemic and nonischemic groups were compared and merged, identifying only those genes common to both lists with $p < 0.05$. Genes in italics represent those reciprocally regulated in ischemic versus nonischemic patients following left ventricular assist device support. Gene names are presented as listed by the Affymetrix software. Parenthetical alias gene names were obtained from the Prosite data base in comparison with GenBank, using the CELL suite from Incellico.
patients before LVAD support was more closely related to the post-LVAD transcriptional profile than that of patients with nonischemic, idiopathic dilated cardiomyopathy. Interestingly, a comparison of statistically significant changes in gene expression following LVAD support from the ischemic and nonischemic patients found only 29 genes that were common to both groups. However, of these 29 genes, the expression of 13 was reciprocally regulated in ischemic versus nonischemic samples. Hence, the regulation of only 16 genes was truly shared between groups (Table 3). Furthermore, nonischemic patients demonstrated a significant downregulation of several genes, as previously described following LVAD support, including BNP (16,17), collagen (18,19), interleukin-8 (22), and matrix metalloproteinase (28). Conversely, ischemic patients showed upregulation of interleukin-8 and no significant regulation of the other genes (see paired t test data in supplemental data of Tables 2 and 3). Interestingly, several hallmark genes of HF, such as β-myosin heavy chain and skeletal α-actin were also significantly downregulated following LVAD support in nonischemic patients, although there was no change or upregulation of these genes in ischemic patients (Fig. 4A). These data were corroborated with Northern blot analysis of these two genes, which were qualitatively similar to the microarray data in terms of expression and significance (Fig. 4B). These data underscore the divergent phenotypic and genomic LVAD-mediated reverse remodeling that occurs in ischemic and nonischemic cardiomyopathies.

**Regulation of metabolic genes.** Finally, genes determined to be significantly regulated following LVAD support in patients, by the paired t test, were clustered according to the biologic process by linking the GenBank identification number with the Gene Ontology data base using the coded electronic life library cross-referencing tool. This clustering demonstrated an enhancement (albeit nonsignificant) in the percentage of metabolic genes changed significantly following LVAD support, as compared with the entire population of genes represented on the microarray (Fig. 5), similar to the previously described regulation of metabolic genes in HF (29,30). A small sample of this type of clustering is presented in Figure 5, along with a table representing the number and percentage of genes in families of biologic function both from the t test analysis and as represented on the entire array (Fig. 5B).

**DISCUSSION**

Herein we have reported our investigation of global changes in gene expression following LVAD support, using a statistically oriented approach in paired human samples, thus providing a host of potentially novel diagnostic and/or therapeutic targets for future investigation. Our current study provides an expanded view of the significant changes in gene expression associated with LVAD support of the failing human heart. Furthermore, the data demonstrate a more homogeneous gene expression profile following LVAD support, as compared with pre-LVAD samples, suggesting that there may be a genomic signature of LVAD-associated reverse remodeling.

**Differential pre- and post-LVAD gene expression.** In a statistically significant manner, we found that of ~7,000 genes, 295 were upregulated and 235 were downregulated following LVAD support. Although the expression of some genes already known to be altered by LVADs were found to be similarly changed in this study, the majority of the differential gene expression found consisted of genes not previously known to be affected by LVAD support, including several genes without a clear connection to cardiomyopathy. Interestingly, when clustering differential post-LVAD gene expression according to functional processes, a
high percentage of genes involved in metabolism were found to have significantly altered expression. These included genes involved in deoxyribonucleic acid, RNA, and protein metabolism, as well as general energy pathways. Thus, it appears that while the heart is mechanically unloaded, the process of reverse remodeling is associated with significant, active changes in cellular metabolic pathways.

Our results demonstrate that pre- and post-LVAD samples have distinct and statistically significant segregating genomic footprints, providing evidence that the reverse remodeling process and associated phenotypic changes after LVAD support are accompanied by distinct changes in gene expression. It is probable that among the 530 genes that were regulated in a statistically significant manner following LVAD support are genes indicative and predictive of the reverse remodeling process. Of particular interest are genes that previously have not been considered in the setting of HF. Further investigation of these genes will be required to understand their specific roles in both cardiomyopathy and the reverse remodeling process. Importantly, these genes may provide a host of novel targets for future diagnostic or therapeutic indications.
Genomic patient stratification concordant with HF etiology. Previously, several investigators have suggested that patients with nonischemic cardiomyopathy undergo more extensive reverse remodeling than ischemic patients following LVAD support (6,31). Our data certainly support that notion, demonstrating much more extensive changes in gene expression following LVAD support in nonischemic versus ischemic patients. Indeed, it seems intuitive that the remaining viable myocardium in ischemic patients is most probably much more functional than the more globally dysfunctional tissue found in idiopathic dilated myocardium. Therefore, a heart that has undergone extensive remodeling over time (i.e., idiopathic dilated cardiomyopathy) would undergo dramatic changes following mechanical support. In contrast, the myocardium within an ischemic heart generally composed of both scar tissue and viable, functional myocardium may undergo less dramatic changes under mechanical support. Importantly, our segregation of the patients’ genomic response to LVAD support was obtained in blinded fashion, underscoring the clearly divergent, HF etiology-specific changes in gene expression. Interestingly, our serendipitous inclusion of three ischemic and three nonischemic patients may provide the explanation for why we were unable to find all the genes previously associated with LVAD support, as the majority of these studies have been carried out strictly in nonischemic patients. When considering vast human heterogeneity, it is striking that a small sample size could clearly distinguish the difference in HF etiology by myocardial gene expression. Subsequently, we determined differential LVAD-mediated regulation of gene expression in these two divergent types of HF. Importantly, we found significant differences in gene expression resulting from LVAD support when starting with either a failing ischemic or nonischemic heart.

Surprisingly, among the expression of hundreds of genes that were significantly regulated, only 16 were similarly regulated in a significant fashion. Also of interest was the fact that in the paired t test analysis, data from previous pre-versus post-LVAD studies of four individual genes was corroborated in the nonischemic patients, whereas there was no corroboration in the ischemic patients, underscoring the divergent, HF etiology-specific response to LV unloading. Interestingly, several hallmark genes of HF, such as skeletal α-actin and β-myosin heavy chain, were downregulated following LVAD support in the nonischemic patients, whereas there was no change or a trend toward upregulation of these genes in the ischemic patients. This disparity may explain, in part, why less hallmark genes of HF previously described were found to be significantly downregulated following LVAD support in the complete cohort of six patients. Our findings underscore the well-described phenotypic distinction between these two types of cardiomyopathy and suggest different mechanisms of LVAD-mediated recovery dependent on HF etiology.

Potentially predictive value of the study. Several groups have also envisioned the use of LVADs as a potential “bridge to recovery," where the extent of recovery in certain patients would be sufficient to allow weaning from LVAD support and obviate the necessity of transplantation (32–35). However, there has been substantial variability in the reported patient response to LVAD weaning. Importantly, the investigators of the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart failure (REMATCH) trial recently published exciting results demonstrating that long-term LVAD support provides significantly improved morbidity and mortality, as compared with optimized medical management, in patients with end-stage heart failure who are ineligible for transplantation (36). One of the central challenges of long-term LVAD support, or particularly the bridge to recovery, is choosing the “correct” patient(s) for these procedures, partly due to a relative paucity of data on the mechanisms of reverse remodeling. A limited number of methods have been proposed to “predict" the patient’s response to long-term LVAD or weaning, such as pre-LVAD creatinine levels (10), decreased circulating levels of BNP (17), duration of illness (2), nonischemic cardiomyopathy (6,31,32), decreased bilirubin levels (37), age (37), and pre-implant ventilation (37). However, these reports also urge caution in predicting an outcome based on a single factor. Along with the development of predictive methodologies, serial determination of cardiac function (and recovery) during LVAD support, determination of optimal support modalities, medical therapies, and weaning strategies will need to be developed. Although the salutary effect of LVAD support is evident, the molecular mechanisms underlying reverse remodeling have not been well described.

Our study demonstrates that the phenotypic changes that occur following LVAD support are associated with genotypic changes in the form of significantly altered myocardial gene expression profiles. We believe combining our methods for statistically analyzing genomic profiles reported herein with a variety of data, including but not limited to clinical history and HF etiology, may provide promising diagnostic tools to determine, a priori, which patients would most likely respond favorably to long-term LVAD or weaning from the device in lieu of heart transplantation. Indeed, studies are now attempting to use multimodal data (including microarray and clinical data) to predict the cellular or patient response to pharmacological and/or surgical intervention in several disease states, particularly cancer (38,39).

Herein we have described our ability to statistically distinguish patients’ LVAD status and HF etiology using oligonucleotide microarrays. Furthermore, we have identified differential expression of numerous genes significantly associated with mechanical unloading of the failing heart, both in general and specific to ischemic or nonischemic HF etiology. These data and statistical methods of microarray analysis provide substantial insight into the potential mechanisms of reverse remodeling and, with further study and development, may facilitate the prognostic prediction of the individual patient response to LVAD support.
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