Transcriptomal analysis of failing and nonfailing human hearts

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Heart failure is a multifactorial disease that may result from different initiating events. To contribute to an improved comprehension of normal cardiac function and the molecular events leading to heart failure, we performed large-scale gene expression analysis of failing and nonfailing human ventricle. Our aim was to define and compare expression profiles of 4 specific pathophysiological cardiac situations: 1) left ventricle (LV) from nonfailing heart; 2) LV from failing hearts affected by dilated cardiomyopathy (DCM); 3) LV from failing hearts affected by ischemic CM (ICM); 4) right ventricle (RV) from failing hearts affected by DCM or ICM. We used oligonucleotide arrays representing ~12,000 human genes. After stringent numerical analyses using several statistical tests, we identified 1,306 genes with a similar expression profile in all 4 cardiac situations, therefore representative of part of the human cardiac expression profile. A total of 95 genes displayed differential expression between failing and nonfailing heart samples, reflecting a reversal to developmental gene expression, dedifferentiation of failing cardiomyocytes, and involvement of apoptosis. Twenty genes were differentially expressed between failing LV and failing RV, identifying possible candidates for different functioning of both ventricles. Finally, no genes were found to be significantly differentially expressed between failing DCM and failing ICM LV, emphasizing that transcriptomal analysis of explanted hearts results mainly in identification of expression profiles of end-stage heart failure and less in determination of expression profiles of the underlying etiology. Taken together, our data resulted in identification of putative transcriptomal landmarks for normal and disturbed cardiac function.

The evolution of methods that enable large-scale expression analyses, like serial analysis of gene expression (SAGE), in situ analysis of expressed sequence tag (EST) databases, cDNA microarrays, and oligonucleotide microarrays, allows researchers to establish organ- or pathology-specific transcriptional profiles. This may lead to the formation of a custom collection of genes, applicable to a specific pathology. These custom collections will allow high-throughput expression analyses of large quantities of samples. Therefore, besides a better understanding of pathological processes, these studies may eventually open the way to improved diagnosis and new treatment strategies for certain human diseases. Most of these methods have been applied to studies of human cancer. Cardiovascular diseases, which also represent a major cause of morbidity and mortality in industrialized countries, have been studied only to a limited extent by these methods. The main reason for this discrepancy is that it is relatively easy to obtain tumor material, whereas human cardiac tissue is, evidently, much harder to come by.

Heart failure is a complex clinical syndrome, which is defined as the development and progression of left ventricle (LV) remodeling (16). It constitutes the endpoint of many different cardiac diseases and is therefore considered to be multifactorial. Two cardiac diseases that may eventually lead to heart failure are dilated (DCM) and ischemic (ICM) cardiomyopathy. Although, or, possibly, resulting from the fact that, clinical treatment for cardiac diseases has improved over the last few decades, heart failure morbidity and mortality have increased (14). To obtain a better understanding of the factors involved in the development of heart failure, we need to improve our knowledge of the disturbed molecular pathways in cardiac pathology. The recently developed techniques, which enable the study of the cardiac transcriptome, may provide us with a tool toward this goal. Expression analyses will allow the identification of a molecular portrait of the human heart, which will indicate the transcriptional requirements for cardiac function. Thus far, studies toward this goal have been based solely on analyses of EST libraries (5, 21). Recently, a report describing the application of SAGE for global expression analysis of mouse heart has been published (2). In addition, large-scale gene expression analyses have been used to detect aberrant expression in cardiac pathologies. The effect of ICM on gene expression levels has been stud-
DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; LV and RV, left and right ventricle, respectively; NF, nonfailing; NA, not available; AC, acebutolol; AM, amiodarone; CP, captopril; CR, carvedilol; DB, dobutamine; DG, digoxin; DP, dopamine; EN, enoximone; EP, epinephrine; FR, furosemide; HCT, hydrochlorothiazide; LS, losartan; ML, molsidomine; NC, nicorandil; SP, spironolactone; CF, cystic fibrosis.

Our goal was to comparatively analyze gene expression in failing and nonfailing human hearts using oligonucleotide microarrays (Affymetrix) containing ~12,000 human genes, representative of a substantial proportion of the human transcriptome. Expression profiles of well-defined cardiac tissues (failing/nonfailing, DCM/ICM, LV/RV) were compared to identify genes expressed at a similar level in all cardiac tissues and to identify genes differentially expressed between the same tissues. In this way we aimed to identify transcriptomal landmarks for normal and disturbed cardiac function.

**MATERIALS AND METHODS**

*Human cardiac tissue.* Left (LV) and right (RV) ventricle tissue was obtained from ICM- and DCM-affected explanted hearts from patients diagnosed with end-stage heart failure, who underwent a heart transplantation. Hearts that were used to obtain RV tissue were selected based on the following criteria: no RV dilation, normal RV ejection fraction, and normal pulmonary blood pressure. Nonfailing LV tissue was obtained from one cystic fibrosis (CF) patient (patient NF LV) who underwent a heart-lung transplantation. This patient did not show any sign of heart failure. Chest X-ray, ECG, and echocardiography revealed no cardiac abnormalities. Since both pathological and control hearts were obtained from patients who underwent a heart transplantation, the clinical settings (which may influence gene expression) were comparable for both situations. To determine whether patient NF LV represented a valid control for our study, 11 additional NF heart samples were obtained. These samples included LV from two CF patients who underwent a heart-lung transplantation (patients NF01 and NF05), two commercial RNA samples from human adult heart obtained from Stratagene (NF03 and NF04), and seven LV samples from general organ donors (NF02, NF06, NF07, NF08, NF09, NF10, and NF11) undergoing pulmonary and aortic valve transplantation surgery. Before explantation of the hearts, the patients did not receive any medication except for dobutamine, furosemide, and plasma expanders. The experimental protocol complied with the Declaration of the World Medical Association proclaimed in Helsinki and was approved by the Ethical Review Board of the Medical Faculty of the University of Szeged (No. 51–57/1997 OEj). See Tables 1 and 2 for patient characteristics.

**Expression profiling.** Total RNA was isolated separately from each cardiac tissue using TRIzol Reagent (Life Technologies). RNA was DNase treated, and quality was assessed by migration on a 1% agarose gel and RT-PCR using primers for ß-actin. Absence of DNA contamination was verified by PCR.

**Table 1. Characteristics of patients analyzed by microarray and real-time PCR analysis**

<table>
<thead>
<tr>
<th>Total RNA Pool:</th>
<th>DCM LV</th>
<th>ICM LV</th>
<th>Failing Hearts</th>
<th>DCM/ICM RV</th>
<th>Nonfailing Heart (NF LV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient:</td>
<td>D1</td>
<td>D2</td>
<td>I1</td>
<td>I2</td>
<td>I3</td>
</tr>
<tr>
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<td>45</td>
<td>58</td>
<td>65</td>
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<td>66</td>
</tr>
<tr>
<td>Sex</td>
<td>male</td>
<td>male</td>
<td>male</td>
<td>male</td>
<td>male</td>
</tr>
<tr>
<td>Pathology</td>
<td>DCM</td>
<td>DCM</td>
<td>ICM</td>
<td>ICM</td>
<td>ICM</td>
</tr>
<tr>
<td>Tissue</td>
<td>LV</td>
<td>LV</td>
<td>LV</td>
<td>LV</td>
<td>RV</td>
</tr>
<tr>
<td>Ejection</td>
<td>23</td>
<td>13</td>
<td>23</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>fraction, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>CP, DG, AM, CP, AM, CP, DB, FR, FR, FR, NC, FR, AM, CP, DB, AC, AM, CR, DG, FR, AM, CP, AM, DB, EN, none</td>
<td></td>
<td></td>
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Table 2. Characteristics of patients analyzed by real-time PCR only

<table>
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<th>Sex</th>
<th>Pathology</th>
<th>Cause of Death</th>
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<tbody>
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<td>CF</td>
<td>CVA</td>
</tr>
<tr>
<td>NF02</td>
<td>53</td>
<td>female</td>
<td></td>
<td>unknown</td>
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<tr>
<td>NF03</td>
<td>72</td>
<td>male</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>NF04</td>
<td>63</td>
<td>male</td>
<td>CF</td>
<td>CVA</td>
</tr>
<tr>
<td>NF05</td>
<td>32</td>
<td>male</td>
<td>CF</td>
<td>CVA</td>
</tr>
<tr>
<td>NF06</td>
<td>57</td>
<td>male</td>
<td>CF</td>
<td>CVA</td>
</tr>
<tr>
<td>NF07</td>
<td>18</td>
<td>female</td>
<td></td>
<td>accident</td>
</tr>
<tr>
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<td>accident</td>
<td>CVA</td>
</tr>
<tr>
<td>NF09</td>
<td>51</td>
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<td>CVA</td>
<td>CVA</td>
</tr>
<tr>
<td>NF10</td>
<td>42</td>
<td>male</td>
<td>CVA</td>
<td>CVA</td>
</tr>
<tr>
<td>NF11</td>
<td>43</td>
<td>male</td>
<td>CVA</td>
<td>CVA</td>
</tr>
</tbody>
</table>

NF03 and NF04 represent commercial human adult heart RNA samples obtained from Stratagene. For these samples no information is available about cause of death or type of tissue used for RNA extraction. All other NF samples were obtained from LV; CF, cystic fibrosis; CVA, cerebrovascular accident.
using the same primers. Three pooled samples were prepared: DCM LV containing equal amounts of total RNA from LV from patients D1 and D2, ICM LV containing equal amounts of total RNA from LV from patients D3, D4, I2, I3, I4, and I5. Nonfailing LV was obtained from patient NF LV (Table 1). Target preparation, hybridization to the Affymetrix HG-U95A array, and detection was performed as previously described (11). All hybridizations were performed in duplicate on the four samples described above, by dividing the prepared target over two arrays.

Data analysis: genes similarly expressed in the four cardiac samples. Analysis of Affymetrix microarrays was performed using the Affymetrix GeneChip software (version 3.3) as described previously (27). Figure 1 displays the level of intra-experimental reproducibility for the four pairs of duplicate experiments. The determination of the transcriptional profile common for the 4 cardiac samples was performed as follows: the average difference (Avg Diff) values on all 8 arrays were normalized by setting the mean Avg Diff of each array at 5,000. All genes (probe sets) that had received an "absent" call for all 8 arrays and all genes with an average Avg Diff value of <500 (for the 8 hybridizations) were not taken into consideration for the transcriptional profile. The 2,031 remaining genes were submitted to 4 statistical tests of increasing stringency using SAS Software (Bonferroni, Newman-Keuls, Ryan-Einot-Gabriel-Welsch, least significant difference, \( \alpha = 0.05 \)) to filter out those genes with a similar expression level in all tissues analyzed, i.e., genes with a similar average Avg Diff value (for the 2 duplicate hybridizations) for the 4 tissue samples. Only those genes that passed at least three of the statistical tests were included in the human cardiac transcriptional profile, since their expression level did not seem to be dependent on the (pathological) origin of the tissue.

Data analysis: genes differentially expressed between the four cardiac samples. The identification of genes differentially expressed in the four cardiac samples (DCM LV, ICM LV, DCM/ICM RV, and NF LV) was performed in a fashion similar to that described previously (11). Global scaling was performed to a constant value of 800, as recommended by the manufacturer. The following comparisons of data sets were performed, using the GeneChip software: DCM LV vs. NF LV, ICM LV vs. NF LV, DCM/ICM RV vs. NF LV, DCM LV vs. DCM/ICM RV, ICM LV vs. DCM/ICM RV, and DCM LV vs. ICM LV. Since all hybridizations had been performed in duplicate, we obtained four data sets for each of the six comparisons. Statistically significant differential genes were selected using the following procedure. Genes that had received an "absent" call for all eight chips were eliminated from further analysis. For the remaining genes \((n = 6,585)\) we transformed the "fold change" values into \(\log_2(\text{ratio})\) values (4 values for each comparison). On these values we performed significance analysis of microarrays (SAM) (44). Using the "one class" option and setting the false discovery rate at 3%, we tested whether the mean of the fold expression ratios within one comparison differed from zero. The genes that where then labeled as "significant" were further analyzed using Affymetrix criteria. We retained those genes that displayed a (marginal) increase or decrease and a fold change \(>2.0\) or less than \(-2\) (or \(\log_2(\text{ratio}) > 1.0\) or less than \(-1.0\)) in all four data sets from one comparison. In addition, we retained those genes with a fold change \(>2.0\) or less than \(-2\) when in at least one of the neighboring comparisons the "difference call" was consistently (marginal) increase or decrease.

Fig. 1. Intra-experimental reproducibility of normalized signals of hybridization to the 12,626 probe sets on the Affymetrix chips in the duplicate experiments. All points that are located between the dotted lines represent probe sets with an average difference (Avg Diff) ratio between the duplicate experiments of \(>0.5\) and \(<2.0\). DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; LV and RV, left and right ventricle, respectively; NF, nonfailing.
decrease for the four data sets. Neighboring comparisons are those in which the denominator is identical. Genes with a ratio between the duplicate normalized Avg Diff values < 0.5 or > 2.0 were not considered for differential analysis.

Real-time PCR. Ten genes (11 transcripts) were selected for quantitative analysis by real-time PCR using SYBR Green I dye (PE Biosystems). Genes and primers were: cytokine inducible nuclear protein (forward CTGTTATTTTTACTGCCAGCT, reverse CATTGTTTCCTTTCCTTG), pyruvate dehydrogenase kinase isoform 4 (forward CTCCAAAACAAACGACAGCAA, reverse ACTCACTCCTTTCTTATCAA), complement protein component C7 (forward ATGCTCGTCTCCCAACTCCTGA, reverse TGACACATCCTAAAACCCCAAAGAG), δ sleep inducing peptide (forward CCTTGTTGATCCCTCCAC, reverse ATGCCAAGCCACCATTCA), ANF (forward CATTTGTGTTCATCTTGTTGCCA, reverse GAGGCGAGGAGTCACCATC), BNP (forward ACCGCAAAATGGCTCTCCTA, reverse TGTTGAATCAGAAGCAGGTGCTC), AF1q (forward CCTACTCCTTTGACTTTAGTCC, reverse TAGCTGAAACTGCTGCCAC), SH3BGR (forward GTGAAGACTGTTTATGCATAC, reverse CACAGAGAACTTCCATGCCTTG), FLRG (forward AGACCCAGACTCCAGGAC, reverse TGTGTTTACAGGCCAGCAG), SLIMMER (forward CCGTGTGTTGATCCCTCCAC, reverse ATGCCAAGCCACCATTCA), hypoxanthine phosphoribosyltransferase (HPRT) was taken as internal standard. Two types of real-time PCR analyses were performed. First, we wanted to confirm the results obtained by microarray analysis. For this we used the same cardiac samples that had been analyzed by the arrays: DCM LV, ICM LV,
DCM/ICM RV, and NF LV. Gene expression ratios were calculated for DCM LV vs. NF LV, ICM LV vs. NF LV, and DCM/ICM RV vs. NF LV. The second type of real-time PCR analysis was performed to evaluate our choice of control tissue in the microarray experiments. We determined expression ratios of the above mentioned genes in 11 additional nonfailing hearts vs. NF LV. All PCR reactions were performed in two experiments, each containing duplicate reactions (n = 4 for each expression value).

**RESULTS**

**Human heart sampling and global view.** Our goal was to compare gene expression profiles in human failing and nonfailing LV and RV, using Affymetrix HG-U95A arrays containing 12,626 probe sets. In duplicate experiments we determined expression profiles of LV from one CF patient (patient NF LV), of a pooled sample of LV from two DCM-affected failing hearts (DCM LV), of a pooled sample of LV from two ICM-affected failing hearts (ICM LV), of a pooled sample of RV from two ICM-affected failing hearts (ICM RV), of a pooled sample of RV of two DCM-affected failing hearts (DCM RV) and NF LV. All PCR reactions were performed in two experiments, each containing duplicate reactions (n = 4 for each expression value).

**Genes similarly expressed in the four cardiac samples.** We identified 1,306 probe sets (corresponding to 1,258 unique genes) that displayed similar (i.e., statistically non-different) Avg Diff values in all 4 samples analyzed. The similarity of the Avg Diff values of these probe sets indicated that the expression levels of the corresponding genes were not significantly influenced by pathology, age, or left vs. right ventricular origin. (Complete data are available on http://www.ifr26.nantes.inserm.fr/composantes/u533/people/team1b/publi/affylogin.htm) Since the redundant genes were represented on the array by different probe sets, which, in some cases, performed differently, we did not exclude this redundancy (e.g., acid ceramidase, http://www.ifr26.nantes.inserm.fr/composantes/u533/people/team1b/publi/affylogin.htm). This explains why some accession numbers appear twice in the profile on http://www.ifr26.nantes.inserm.fr/composantes/u533/people/team1b/publi/affylogin.htm. The distribution of the level of expression of these 1,306 genes selected on the basis of similar expression levels in all cardiac samples analyzed. Level of expression is represented as a percentage of the cumulative expression of the 1,306 genes. The Avg Diff values representing the borders between highly, moderately, and weakly expressed genes are indicated by arrows.

Fig. 3. Distribution of the level of expression of 1,306 genes selected on the basis of similar expression levels in all cardiac samples analyzed. Level of expression is represented as a percentage of the cumulative expression of the 1,306 genes. The Avg Diff values representing the borders between highly, moderately, and weakly expressed genes are indicated by arrows.
communication and cell structure and motility. Only a few genes are involved in cell division, and these concern only those weakly or moderately expressed. The highly expressed genes (Table 3) contained several typical cardiac genes, like \( /H9251\)-cardiac actin, ventricular myosin alkali light chain, and phospholamban. Most of the ribosomal proteins were moderately expressed, whereas transcription factors were mostly weakly expressed. Figure 4 shows the distribution of the genes over the functional categories in the three groups of genes at different level of expression. This distribution differed between the highly and the weakly expressed genes. Genes involved in metabolism were more strongly represented among the highly expressed genes (Table 3). Furthermore, this group contained relatively more genes involved in cell structure and motility and fewer genes involved in cell signaling and communication.

**Genes differentially expressed between the four cardiac samples.** The sequential application of SAM analysis and Affymetrix criteria to the expression ratios obtained from the comparisons among the four specific cardiac pathophysiological situations led to a stringent, statistically valid selection of subsets of differentially expressed genes. We identified 95 genes differentially expressed in failing vs. nonfailing heart (Tables 4–6), 20 genes differentially expressed between failing LV and failing RV (Table 7), and 0 genes differentially expressed between DCM LV and ICM LV. The subsets of differentially expressed genes were composed as follows.

Differential genes between failing heart and nonfailing heart.** In Tables 4–6 we grouped together genes showing differential expression in either all three (group A, Table 4), or in two of three (group B, Table 5), or in only one (group C, Table 6) of the failing heart samples compared with NF LV. Some genes that did not show consistent differential expression in all four comparisons according to the Affymetrix criteria, but that were significantly differential according to SAM and had a \( \log_2(\text{ratio}) > 1.0 \) or less than \(-1.0\), were.

Table 3. Genes highly expressed in human heart

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Description</th>
<th>Expression, %</th>
<th>Functional Category</th>
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<td>F27891</td>
<td>cytochrome c oxidase subunit VIa polypeptide 2</td>
<td>0.83</td>
<td>M</td>
</tr>
<tr>
<td>AA152406</td>
<td>cytochrome c oxidase subunit VIIa polypeptide 1</td>
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<tr>
<td>D55654</td>
<td>cytosolic malate dehydrogenase</td>
<td>0.72</td>
<td>M</td>
</tr>
<tr>
<td>M33197</td>
<td>glyceraldehyde-3-phosphate dehydrogenase</td>
<td>0.71</td>
<td>M</td>
</tr>
<tr>
<td>AA426364</td>
<td>ATP synthase, H+ transporting, mitochondrial F0 complex, subunit e</td>
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<tr>
<td>N47307</td>
<td>NADH dehydrogenase (ubiquinone) 10 subcomplex, 1</td>
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<tr>
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<td>glyceraldehyde-3-phosphate dehydrogenase</td>
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<td>M</td>
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<td>cytochrome c oxidase subunit VIc</td>
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<td>T79616</td>
<td>ubiquinol-cytochrome c reductase binding protein</td>
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</table>

Expression is given as a percentage of the cumulative expression of the 1,306 genes present in the transcriptional profile. U, unclassified; CS/M, cell structure and motility; M, metabolism; G/PE, gene and protein expression; C/OD, cell and organism defense; and CS/C, cell signaling and communication.
considered as being differentially expressed. For example, cyclin-dependent kinase inhibitor 1A (CDKN1A), showed an average log$_2$(ratio) of 4.49, 4.46, and 3.82 in DCM-affected LV, ICM-affected LV, and DCM/ICM-affected RV, respectively. Even though CDKN1A did not show consistent increased expression in DCM-affected LV according to the Affymetrix criteria, we did classify this gene as belonging to group A. This approach was only used for those genes that did pass all criteria in at least one of the samples tested. In addition to a classification according to the main functional categories, we attempted to assign the differential genes to the different molecular circuits known to be affected in heart failure. We identified 24 genes (group A) showing increased expression in all failing heart samples. In this group we identified eight genes involved in signaling pathways. Among those were the natriuretic factors ANF and BNP, which are involved in vascular homeostasis. Seven genes in this group are involved in biomechanical functions. These genes encode proteins that are components of the sarcomere (e.g., embryonic myosin alkaline light chain), the cytoskeleton (e.g., α-actinin), or the extracellular matrix (e.g., versican). Furthermore, several genes implicated in energy metabolism, oxidative stress, and inflammation showed increased expression in failing LV and RV. In addition, we identified a gene with unknown function in this group (AF1q). Group A did not contain any downregulated genes, since they failed to pass the SAM analysis for all three failing heart samples.

The next group of differentially expressed genes, belonging to group B, concerns those whose expression changed in the LV of DCM- and ICM-affected hearts but not in the RV. Therefore, the expression of these genes changed only in the part of the heart that is affected the most by the disease. This group, consisting of 19 upregulated genes, also contained a substantial amount of genes (a total of 6) involved in biomechanical functions. Most interesting were those genes whose log$_2$(ratio) was close to 0 in the failing RV sample and >1.0 in the failing LV samples [e.g., transglutaminase (TGase) and α-tubulin isotype H2-α]. It should be noted that for some of the genes the log$_2$(ratio) value is not listed because the ratio between the Avg Diff values of the two duplicate hybridizations were outside of the range 0.5–2.0, and results were therefore considered to be unreliable [Tables 5–7; not reliable (NR)]. The classification of those genes in this group should therefore be regarded as ambiguous. Only 5 genes were upregulated in DCM-affected LV and DCM/ICM-affected RV only, whereas 20 genes were differentially expressed in ICM-affected LV and DCM/ICM-affected RV only. A logical explanation for genes assigned to this last group could be that they are differentially expressed in ICM-affected hearts in general, and since the DCM/ICM-affected RV pool consisted mainly of tissue from ICM-affected hearts the same genes would be found to be differentially expressed. However, several genes were upregulated to a higher extent in the RV sample than in the ICM-affected LV sample (e.g., nidogen and TP53BP2) and might therefore represent RV-related events.

We found 27 genes to be differentially expressed in only 1 of the 3 failing heart samples analyzed (group C). Two of these were found only in DCM-affected LV (heat shock 70-kDa protein 5 and cytokine inducible nuclear protein). Seventeen genes were deregulated only in ICM LV, with 13 genes up- and 4 genes downregulated. Three of the upregulated genes are involved in signaling pathways: Ras-like protein Tc21, cAMP-responsive element modulator (CREM), and A kinase anchor protein 2 (AKAP2). One of the genes displaying a clear ICM-specific upregulation was pyruvate dehydrogenase kinase isoform 4, a gene involved in glucose metabolism. Of the eight genes differentially expressed specifically in failing RV, two genes were downregulated and six genes were upregulated. The two downregulated genes could be reduced to one nonredundant
Table 4. Genes differentially expressed in failing hearts vs. nonfailing heart: Group A

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Description</th>
<th>DCM LV vs. NF LV</th>
<th>ICM LV vs. NF LV</th>
<th>DCM/ICM RV vs. NF LV</th>
<th>Functional Category</th>
<th>Molecular Circuit</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL021155</td>
<td>brain natriuretic protein</td>
<td>7.24 [+ +</td>
<td>7.80 [+ +</td>
<td>7.41 [+ +</td>
<td>CS/C</td>
<td>Signaling</td>
</tr>
<tr>
<td>AL021155</td>
<td>atrial natriuretic factor</td>
<td>4.83 [+ +</td>
<td>4.59 [+ +</td>
<td>4.12 [+ +</td>
<td>CS/C</td>
<td>Signaling</td>
</tr>
<tr>
<td>X51345</td>
<td>JUN-B</td>
<td>3.40 [+ +</td>
<td>3.40 [+ +</td>
<td>2.40 [+ +</td>
<td>G/PE</td>
<td>Signaling</td>
</tr>
<tr>
<td>S37730</td>
<td>insulin-like growth factor binding protein-2</td>
<td>2.87 [+ +</td>
<td>3.20 [+ +</td>
<td>2.27 [+ +</td>
<td>CS/C</td>
<td>Signaling</td>
</tr>
<tr>
<td>Z34724</td>
<td>polyA site DNA</td>
<td>2.06 [+ +</td>
<td>2.04 [+ +</td>
<td>3.39 [+ +</td>
<td>G/PE</td>
<td>Signaling</td>
</tr>
<tr>
<td>AF060568</td>
<td>promyelocytic leukemia zinc finger protein</td>
<td>2.33 [+ +</td>
<td>2.07 [+ +</td>
<td>1.22 [+ +</td>
<td>G/PE</td>
<td>Signaling</td>
</tr>
<tr>
<td>X78947</td>
<td>connective tissue growth factor</td>
<td>1.65 [+ +</td>
<td>2.10 [+ +</td>
<td>1.75 [+ +</td>
<td>CS/C</td>
<td>Signaling</td>
</tr>
<tr>
<td>Z37976</td>
<td>latent TGF-β binding protein</td>
<td>1.40 [+ +</td>
<td>2.53 [+ +</td>
<td>1.31 [+ +</td>
<td>CS/C</td>
<td>Signaling</td>
</tr>
<tr>
<td>X13839</td>
<td>vascular smooth muscle α-actin</td>
<td>1.76 [+ +</td>
<td>1.94 [+ +</td>
<td>1.19 [+ +</td>
<td>CS/M</td>
<td>Biomechanics-Cytok</td>
</tr>
<tr>
<td>X5804</td>
<td>α-actinin</td>
<td>1.53 [+ +</td>
<td>1.63 [+ +</td>
<td>1.63 [+ +</td>
<td>CS/M</td>
<td>Biomechanics-Cytok</td>
</tr>
<tr>
<td>M95178</td>
<td>non-muscle α-actinin</td>
<td>1.46 [+ +</td>
<td>1.89 [+ +</td>
<td>1.32 [+ +</td>
<td>CS/M</td>
<td>Biomechanics-Cytok</td>
</tr>
<tr>
<td>D17408</td>
<td>calpain</td>
<td>2.77 [+ +</td>
<td>3.95 [+ +</td>
<td>3.41 [+ +</td>
<td>CS/M</td>
<td>Biomechanics-Sarcomere</td>
</tr>
<tr>
<td>X58851</td>
<td>embryonic myosin alkaline light chain</td>
<td>2.79 [+ +</td>
<td>3.36 [+ +</td>
<td>3.14 [+ +</td>
<td>CS/M</td>
<td>Biomechanics-Sarcomere</td>
</tr>
<tr>
<td>AF013570</td>
<td>smooth muscle myosin heavy chain SM2</td>
<td>2.32 [+ +</td>
<td>2.79 [+ +</td>
<td>1.68 [+ +</td>
<td>CS/M</td>
<td>Biomechanics-Sarcomere</td>
</tr>
<tr>
<td>AF001548</td>
<td>myosin, heavy polypeptide 11, smooth muscle</td>
<td>1.71 [+ +</td>
<td>2.01 [+ +</td>
<td>1.18 [+ +</td>
<td>CS/M</td>
<td>Biomechanics-Sarcomere</td>
</tr>
<tr>
<td>X15998</td>
<td>chondroitin sulphate proteoglycan versican</td>
<td>1.69 [+ +</td>
<td>2.54 [+ +</td>
<td>1.44 [+ +</td>
<td>CS/M</td>
<td>Biomechanics-EM</td>
</tr>
<tr>
<td>Y14737</td>
<td>immunoglobulin λ heavy chain</td>
<td>1.68 [+ +</td>
<td>3.08 [+ +</td>
<td>2.87 [+ +</td>
<td>C/OD</td>
<td>Inflammation</td>
</tr>
<tr>
<td>M65292</td>
<td>factor H homolog</td>
<td>2.02 [+ +</td>
<td>2.27 [+ +</td>
<td>1.25 [+ +</td>
<td>C/OD</td>
<td>Inflammation</td>
</tr>
<tr>
<td>U03106</td>
<td>cyclin-dependent kinase inhibitor 1A</td>
<td>4.49 [+ +</td>
<td>4.46 [+ +</td>
<td>3.82 [+ +</td>
<td>CD</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>AA224832</td>
<td>metallothionein 1L</td>
<td>2.59 [+ +</td>
<td>2.17 [+ +</td>
<td>2.93 [+ +</td>
<td>U</td>
<td>Oxidative stress/ Apoptosis</td>
</tr>
<tr>
<td>X79389</td>
<td>GSTT1</td>
<td>1.84 [+ +</td>
<td>2.75 [+ +</td>
<td>2.28 [+ +</td>
<td>M</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td>X16396</td>
<td>NAD-dependent methylene tetrahydrofolate dehydrogenase cyclohydrodase (EC 1.5.1.15)</td>
<td>2.06 [+ +</td>
<td>1.96 [+ +</td>
<td>1.46 [+ +</td>
<td>M</td>
<td>Oxidative/Energy</td>
</tr>
<tr>
<td>J02611</td>
<td>apolipoprotein D</td>
<td>3.02 [+ +</td>
<td>2.00 [+ +</td>
<td>2.47 [+ +</td>
<td>M</td>
<td>Energy</td>
</tr>
<tr>
<td>U16954</td>
<td>A1Pq</td>
<td>3.98 [+ +</td>
<td>4.28 [+ +</td>
<td>4.30 [+ +</td>
<td>U</td>
<td>Unclassified</td>
</tr>
</tbody>
</table>

*Group A*: genes differential in all 3 failing heart samples. For Tables 4–7, “Ratio” represents the mean value of the 4 log2(ratio) values available for every gene (2 for 2 compared vs. 2 duplicates); up arrows (↑) indicate significantly upregulated genes; ratio values with no arrows were not significantly different. Genes that have been assigned a “+” in the “SAM” column, have passed the SAM analysis (significant analysis of microarrays) for significant differential expression. Genes that have been assigned a “−” in the “Affy” column, have passed the Affymetrix criteria for differential expression, as explained in MATERIALS AND METHODS. For log2(ratio) values denoted by “NR” (not reliable) no value is given because the ratio between the duplicate average difference (Avg Diff) values was outside of the range 0.5–2.0. See Tables 5 and 6 for group B (genes differential in 2 failing heart samples) and group C (genes differential in 1 failing heart sample), respectively. Functional categories are as listed in Table 3. Cytosk, cytoskeleton; EM, extracellular matrix.

gene, since both probe sets represented the 18S rRNA gene.

**Differential genes between failing LV and failing RV.**

We found relatively fewer genes (20 in total, Table 7) differentially expressed between failing LV and RV than between failing and nonfailing heart. We divided the 20 differential genes into two major groups: group A, containing all genes differentially expressed in DCM LV vs. DCM/ICM RV and in ICM LV vs. DCM/ICM RV; and group B, containing genes differentially expressed in one of both comparisons. Six genes were upregulated and five genes were downregulated in both DCM LV and ICM LV. These expression changes may reflect a general, nonpathological, difference between LV and RV, but they may also represent a difference in the pathological state of the tissues (in failing hearts, LV function is generally more compromised than RV function). One example is cardiac α-myosin heavy chain (αMHC), a gene that we classified into group A. It has already been reported that αMHC expression does not differ between nonfailing LV and RV (28) and that expression decreases in failing hearts (8, 28, 31) (Table 5, this paper). Therefore, a higher expression in DCM and in ICM LV compared with DCM/ICM RV, as we found in our study, implicates that αMHC expression is more strongly affected in failing RV than in failing LV. Group B consisted of two genes downregulated in DCM LV and seven genes deregulated in ICM LV. A large difference between DCM and ICM LV was noted for c-fos, which showed a log2(ratio) of −0.08 for DCM LV vs. DCM/ICM RV and a log2(ratio) of −1.48 for ICM LV vs. DCM/ICM RV.
### Table 5. Genes differentially expressed in failing hearts vs. nonfailing heart: Group B

<table>
<thead>
<tr>
<th>Description</th>
<th>DCM LV vs. NF LV</th>
<th>ICM LV vs. NF LV</th>
<th>DCM/ICM RV vs. NF LV</th>
<th>Functional Category</th>
<th>Molecular Circuit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ratio</strong></td>
<td><strong>SAM</strong></td>
<td><strong>Affy</strong></td>
<td><strong>Ratio</strong></td>
<td><strong>SAM</strong></td>
<td><strong>Affy</strong></td>
</tr>
<tr>
<td>U08021</td>
<td>nicotinamide N-methyltransferase</td>
<td>2.05</td>
<td>+</td>
<td>+</td>
<td>0.82</td>
</tr>
<tr>
<td>U76702</td>
<td>follistatin-related protein FLRG</td>
<td>1.60</td>
<td>+</td>
<td>+</td>
<td>1.02</td>
</tr>
<tr>
<td>X16302</td>
<td>insulin-like growth factor binding protein 2</td>
<td>1.66</td>
<td>+</td>
<td>1.42</td>
<td>+</td>
</tr>
<tr>
<td>U33632</td>
<td>two P-domain K+ channel TWIK-1</td>
<td>1.62</td>
<td>+</td>
<td>+</td>
<td>0.90</td>
</tr>
<tr>
<td>AJ012737</td>
<td>filamin, muscle isoform</td>
<td>1.77</td>
<td>+</td>
<td>+</td>
<td>1.11</td>
</tr>
<tr>
<td>J00968</td>
<td>adult skeletal muscle α-actin</td>
<td>1.74</td>
<td>+</td>
<td>+</td>
<td>1.12</td>
</tr>
<tr>
<td>K03460</td>
<td>α-tubulin isotype H2-α</td>
<td>1.37</td>
<td>+</td>
<td>+</td>
<td>0.31</td>
</tr>
<tr>
<td>U76456</td>
<td>tissue inhibitor of metalloproteinase 4</td>
<td>2.11</td>
<td>+</td>
<td>+</td>
<td>0.94</td>
</tr>
<tr>
<td>M55153</td>
<td>transglutaminase</td>
<td>1.51</td>
<td>+</td>
<td>+</td>
<td>0.07</td>
</tr>
<tr>
<td>M55153</td>
<td>transglutaminase</td>
<td>1.33</td>
<td>+</td>
<td>+</td>
<td>0.84</td>
</tr>
<tr>
<td>D88674</td>
<td>antizyme inhibitor</td>
<td>1.69</td>
<td>+</td>
<td>+</td>
<td>0.93</td>
</tr>
<tr>
<td>X68277</td>
<td>dual specificity phosphatase 1</td>
<td>2.06</td>
<td>+</td>
<td>+</td>
<td>NR</td>
</tr>
<tr>
<td>L26336</td>
<td>heat shock protein HSPA2</td>
<td>1.34</td>
<td>+</td>
<td>2.50</td>
<td>+</td>
</tr>
<tr>
<td>X07522</td>
<td>truncated form of complement factor H</td>
<td>1.84</td>
<td>+</td>
<td>2.00</td>
<td>+</td>
</tr>
<tr>
<td>Y09836</td>
<td>3UTR of unknown protein</td>
<td>2.37</td>
<td>+</td>
<td>+</td>
<td>2.07</td>
</tr>
<tr>
<td>AB020693</td>
<td>reticulin 4</td>
<td>1.90</td>
<td>+</td>
<td>+</td>
<td>1.02</td>
</tr>
<tr>
<td>AI651806</td>
<td>cystein-rich motor neuron 1</td>
<td>1.80</td>
<td>+</td>
<td>2.03</td>
<td>+</td>
</tr>
<tr>
<td>AP506670</td>
<td>C2H2 zinc finger protein (ZNF 198)</td>
<td>1.51</td>
<td>+</td>
<td>+</td>
<td>1.08</td>
</tr>
<tr>
<td>AF603902</td>
<td>LIM protein SLIMMER</td>
<td>1.38</td>
<td>+</td>
<td>+</td>
<td>0.93</td>
</tr>
<tr>
<td>M18645</td>
<td>Ig rearranged α-chain</td>
<td>1.27</td>
<td>+</td>
<td>+</td>
<td>3.08</td>
</tr>
<tr>
<td>M63438</td>
<td>Ig rearranged γ-chain</td>
<td>1.17</td>
<td>+</td>
<td>+</td>
<td>2.91</td>
</tr>
<tr>
<td>X579009</td>
<td>rearranged immunoglobulin λ light chain</td>
<td>1.55</td>
<td>+</td>
<td>+</td>
<td>2.67</td>
</tr>
<tr>
<td>Y09765</td>
<td>putative GABA receptor ε subunit</td>
<td>0.98</td>
<td>+</td>
<td>+</td>
<td>1.14</td>
</tr>
<tr>
<td>U26336</td>
<td>heat shock protein HSPA2</td>
<td>0.77</td>
<td>+</td>
<td>+</td>
<td>1.14</td>
</tr>
<tr>
<td>U30688</td>
<td>dioxin-inducible cytochrome P450</td>
<td>1.21</td>
<td>+</td>
<td>+</td>
<td>1.14</td>
</tr>
<tr>
<td>M30269</td>
<td>nidogen</td>
<td>1.12</td>
<td>+</td>
<td>+</td>
<td>1.08</td>
</tr>
<tr>
<td>U58334</td>
<td>tumor protein p53 binding protein, 2 (TP53BP2)</td>
<td>1.07</td>
<td>+</td>
<td>+</td>
<td>1.08</td>
</tr>
<tr>
<td>Z84718</td>
<td>DNA sequence from clone 322B1 on 22q11–12</td>
<td>0.93</td>
<td>+</td>
<td>+</td>
<td>1.08</td>
</tr>
<tr>
<td>AB029908</td>
<td>KIAA1075</td>
<td>1.00</td>
<td>+</td>
<td>+</td>
<td>1.02</td>
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<tr>
<td>AF070569</td>
<td>clone 24659 mRNA sequence</td>
<td>0.95</td>
<td>+</td>
<td>+</td>
<td>1.14</td>
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<tr>
<td>D78014</td>
<td>dihydropyrimidinase related protein-3</td>
<td>1.00</td>
<td>+</td>
<td>+</td>
<td>1.14</td>
</tr>
<tr>
<td>AB018283</td>
<td>KIAA0740</td>
<td>1.21</td>
<td>+</td>
<td>+</td>
<td>1.14</td>
</tr>
<tr>
<td>AJ039511</td>
<td>cDNA clone IMAGE-1695674</td>
<td>0.40</td>
<td>+</td>
<td>+</td>
<td>0.70</td>
</tr>
<tr>
<td>D82351</td>
<td>retropseudogene MSSP-1</td>
<td>0.86</td>
<td>+</td>
<td>+</td>
<td>1.05</td>
</tr>
<tr>
<td>AJ537192</td>
<td>SH3BGR</td>
<td>1.23</td>
<td>+</td>
<td>+</td>
<td>1.14</td>
</tr>
<tr>
<td>AL079314</td>
<td>cDNA clone EUROIMAGE 469780</td>
<td>0.60</td>
<td>+</td>
<td>+</td>
<td>1.05</td>
</tr>
<tr>
<td>U28686</td>
<td>RNA binding motif protein 3</td>
<td>0.51</td>
<td>+</td>
<td>+</td>
<td>1.05</td>
</tr>
<tr>
<td>Z20656</td>
<td>cardiac α-myosin heavy chain</td>
<td>1.05</td>
<td>+</td>
<td>+</td>
<td>1.05</td>
</tr>
<tr>
<td>U37408</td>
<td>phosphoprotein CTPB</td>
<td>−1.45</td>
<td>+</td>
<td>+</td>
<td>−1.45</td>
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<tr>
<td>M22430</td>
<td>RASF-A PLAA2</td>
<td>2.28</td>
<td>+</td>
<td>1.30</td>
<td>+</td>
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<tr>
<td>AB005293</td>
<td>perilin</td>
<td>1.94</td>
<td>+</td>
<td>+</td>
<td>1.85</td>
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<tr>
<td>AA128249</td>
<td>fatty acid binding protein 4</td>
<td>1.84</td>
<td>+</td>
<td>+</td>
<td>1.85</td>
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<tr>
<td>V01512</td>
<td>cellular oncogene c-fos</td>
<td>1.51</td>
<td>+</td>
<td>+</td>
<td>0.72</td>
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</tbody>
</table>

**Group B:** Genes differentially expressed in 2 failing heart samples. For details, see legend to Table 4. Functional categories are as listed in Table 3. Up arrows (↑) indicate significantly upregulated genes, and down arrows (↓) indicate significantly downregulated genes; ratio values with no arrows were not significantly different.

**Confirmation of microarray results.** To confirm the validity of our results obtained with the oligonucleotide microarrays, we performed quantitative real-time PCR on 10 genes selected from Tables 4–6: cytokine inducible nuclear protein, pyruvate dehydrogenase kinase isoform 4, complement protein component C7, δ sleep inducing peptide, ANF, BNP, FRLG, SH3BGR, and SLIMMER (Fig. 5). For these genes, the expression
### Table 6. Genes differentially expressed in failing hearts vs. nonfailing heart: Group C

<table>
<thead>
<tr>
<th>Description</th>
<th>DCM LV vs. NF LV</th>
<th>ICM LV vs. NF LV</th>
<th>DCM/ICM RV vs. NF LV</th>
<th>Functional Category</th>
<th>Molecular Circuit</th>
</tr>
</thead>
<tbody>
<tr>
<td>X87949 heat shock protein 5 (HSPA5)</td>
<td>1.35 ↑</td>
<td>0.54</td>
<td>0.48</td>
<td>C/OD Stress</td>
<td></td>
</tr>
<tr>
<td>X83703 cytokine inducible nuclear protein</td>
<td>1.40 ↑</td>
<td>0.65</td>
<td>0.55</td>
<td>U Unclassified</td>
<td></td>
</tr>
<tr>
<td>X15998 chondroitin sulphate proteoglycan versican</td>
<td>0.99</td>
<td>2.02 ↑</td>
<td>1.09</td>
<td>CS/M Biomechanics-EM</td>
<td></td>
</tr>
<tr>
<td>M92642 α-1 type XVI collagen</td>
<td>0.99</td>
<td>1.55 ↑</td>
<td>0.83</td>
<td>CS/M Biomechanics-EM</td>
<td></td>
</tr>
<tr>
<td>J04599 bone proteoglycan I (biglycan)</td>
<td>0.95</td>
<td>1.42 ↑</td>
<td>0.92</td>
<td>CS/M Biomechanics-EM</td>
<td></td>
</tr>
<tr>
<td>X54304 myosin regulatory light chain</td>
<td>1.24</td>
<td>1.22 ↑</td>
<td>1.09</td>
<td>CS/M Biomechanics-Sarcomere</td>
<td></td>
</tr>
<tr>
<td>M95787 SM22 smooth muscle protein, tranfgelin</td>
<td>1.07</td>
<td>1.27 ↑</td>
<td>0.48</td>
<td>CS/M Biomechanics-Cytosk</td>
<td></td>
</tr>
<tr>
<td>HG1111–HT1111 Ras-like protein Tc21</td>
<td>0.86</td>
<td>1.69 ↑</td>
<td>0.19</td>
<td>CS/C Signaling</td>
<td></td>
</tr>
<tr>
<td>S68134 cAMP-responsive element modulator β isoform</td>
<td>NR</td>
<td>1.52 ↑</td>
<td>0.94</td>
<td>G/PE Signaling</td>
<td></td>
</tr>
<tr>
<td>AB023137 A kinase anchor protein 2 (AKAP2)</td>
<td>0.73</td>
<td>1.32 ↑</td>
<td>0.49</td>
<td>CS/C Signaling</td>
<td></td>
</tr>
<tr>
<td>U03688 dioxin-inducible cytochrome P450</td>
<td>1.09</td>
<td>2.26 ↑</td>
<td>1.06</td>
<td>M Energy</td>
<td></td>
</tr>
<tr>
<td>U54617 pyruvate dehydrogenase kinase isoform 4</td>
<td>0.13</td>
<td>1.23 ↑</td>
<td>-0.69</td>
<td>M+CS/C Energy</td>
<td></td>
</tr>
<tr>
<td>X01060 transferrin receptor</td>
<td>1.00</td>
<td>1.32 ↑</td>
<td>0.98</td>
<td>U Iron metabolism</td>
<td></td>
</tr>
<tr>
<td>U51712 cDNA</td>
<td>1.13</td>
<td>1.65 ↑</td>
<td>1.13</td>
<td>U Unclassified</td>
<td></td>
</tr>
<tr>
<td>A1635895 δ sleep inducing peptide, immunoreactor</td>
<td>1.25</td>
<td>1.29 ↑</td>
<td>0.26</td>
<td>U Unclassified</td>
<td></td>
</tr>
<tr>
<td>X92493 STM-7</td>
<td>-0.72</td>
<td>-1.61 ↓</td>
<td>-1.08</td>
<td>CS/C Signaling</td>
<td></td>
</tr>
<tr>
<td>J03507 complement protein component C7</td>
<td>-1.14</td>
<td>-1.46 ↓</td>
<td>-0.68</td>
<td>C/OD Inflammation</td>
<td></td>
</tr>
<tr>
<td>L25286 α-1 type XV collagen</td>
<td>-1.56</td>
<td>-1.42 ↓</td>
<td>-0.56</td>
<td>CS/M Biomechanics-EM</td>
<td></td>
</tr>
<tr>
<td>U87408 clone IMAGE-74593</td>
<td>-1.09</td>
<td>-1.76 ↓</td>
<td>NR</td>
<td>U Unclassified</td>
<td></td>
</tr>
<tr>
<td>U96750 putative tumor suppressor NOEY2</td>
<td>NR</td>
<td>NR</td>
<td>1.86 ↑</td>
<td>+ + U Cell growth</td>
<td></td>
</tr>
<tr>
<td>X67325 interferon, α-inducible protein 27</td>
<td>NR</td>
<td>NR</td>
<td>2.88 ↑</td>
<td>+ + U Unclassified</td>
<td></td>
</tr>
<tr>
<td>L76259 δ-pyruvyltetrahydropterin</td>
<td>0.29</td>
<td>NR</td>
<td>1.92 ↑</td>
<td>+ + U Unclassified</td>
<td></td>
</tr>
<tr>
<td>X84195 acylphosphatase, muscle type isoenzyme</td>
<td>0.52</td>
<td>0.81 ↑</td>
<td>1.86 ↑</td>
<td>+ + U Unclassified</td>
<td></td>
</tr>
<tr>
<td>Y00317 laver microsom al UDP-glucuronosyltransferase</td>
<td>NR</td>
<td>0.90</td>
<td>1.82 ↑</td>
<td>+ + U Unclassified</td>
<td></td>
</tr>
<tr>
<td>X82103 β-COP</td>
<td>0.67</td>
<td>0.73</td>
<td>1.30 ↑</td>
<td>+ + U Unclassified</td>
<td></td>
</tr>
<tr>
<td>U59919 kinesin-associated protein 3</td>
<td>0.76</td>
<td>0.88</td>
<td>1.29 ↑</td>
<td>+ + U Unclassified</td>
<td></td>
</tr>
<tr>
<td>M10098 18S rRNA</td>
<td>1.00</td>
<td>-0.03</td>
<td>-4.02 ↓</td>
<td>+ + G/PE Translation</td>
<td></td>
</tr>
<tr>
<td>M10098 18S rRNA</td>
<td>1.26</td>
<td>0.11</td>
<td>-3.15 ↓</td>
<td>+ + G/PE Translation</td>
<td></td>
</tr>
</tbody>
</table>

**Group C**: genes differential in 1 failing heart sample. For details, see legend to Table 4. Functional categories are as listed in Table 3. Up arrows (↑) indicate significantly upregulated genes, and down arrows (↓) indicate significantly downregulated genes; ratio values with no arrows were not significantly different.

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ratios of failing vs. nonfailing heart determined by PCR were compared with the array data in Tables 4–6. Overall, although the expression ratios were not identical, the up- or downregulation of the 10 genes as determined by microarray analysis was comparable to that determined by real-time PCR. For two genes (pyruvate dehydrogenase kinase isoform 4 in DCM LV and δ sleep inducing peptide in DCM/ICM RV) an upregulation was found using microarrays, whereas real-time PCR showed a downregulation. However, in both cases no significant differential expression was detected by our microarray analysis because of failure to pass Affymetrix and SAM criteria. Since SLIMMER is an isofrom of the skeletal muscle LIM protein 1 (SLIM1) (9), we designed two pairs of primers; one pair specific for SLIMMER, and one pair from the sequence common to both isoforms. Both pairs identified an upregulation of mRNA in failing ventricle. However, the absolute levels of expression of SLIMMER as determined by microarray (high) and real-time PCR (low, data not shown) made us believe that the SLIMMER probe sets on the array also hybridized with SLIM1 mRNA. Indeed, when we checked the exact sequence of the SLIMMER probe set on [http://www.netaffx.com](http://www.netaffx.com), we found that the sequence was in the region common to SLIM1 and SLIMMER.

**Evaluation of the control tissue.** To determine whether NF LV represented a valid control for our microarray study, we measured expression ratios of 10 genes (the same as described above) in 11 additional nonfailing heart samples (NF01–NF11, Table 2) vs. NF LV using real-time PCR. All nonfailing heart samples were obtained from individuals without cardiac disease. Two samples were from CF patients, two samples were obtained as commercially available RNA (Stratagene), and seven samples came from individuals who had died from accidents or cerebrovascular accidents. Ages varied from 18 years to 72 yr, and the series...
DISCUSSION

Tissue sampling. Our goal was to obtain and compare global images of transcription profiles of four well defined pathophysiological situations in human heart. We believed that this would allow an initial selection of genes of interest and could serve as a basis for follow-up studies of large series of individual patients. For this reason we decided to use pooled samples. The reason for the small sizes of the pools was that we included only those patients with similar clinical characteristics and with a phenotype that could be considered as “representative” for the disease, to exclude any excessive biological heterogeneity. A similar approach was recently used by Hwang et al. (22) in an analysis of gene expression profiles of dilated and hypertrophic failing hearts. For the nonfailing sample we chose LV from an explanted heart of a CF patient, for different reasons. The most evident reason is that this patient had been followed closely by clinicians and did not show any sign of heart failure. Although RV dysfunction has been found in adult patients with severe CF, LV abnormalities have not (or very rarely) been associated with this disease (15). Another advantage of our approach was that both types of tissue, failing and nonfailing heart, were obtained under similar clinical circumstances; both types of hearts came from patients who were undergoing heart transplantation. A disadvantage of our approach is that heart/lung transplantation in CF patients is usually performed at a relatively young age compared with heart transplantation in heart failure patients. Therefore there was a substantial age difference between the failing and nonfailing hearts. We cannot exclude that the CF phenotype was responsible for some of the expression changes we identified in failing hearts (2). For the nonfailing sample we chose LV included both female (n = 2) and male (n = 9) patients. Figure 6 shows that for 6 of the 10 genes (cytokine inducible nuclear protein, pyruvate dehydrogenase kinase isofrom 4, complement protein component C7, SH3BGR, FLRG, and ANF) the expression value of NF LV remained within the variation of the 11 individual expression values of NF01–NF11 (complete data available on http://www.ifr26.nantes.inserm.fr/composantes/u533/people/team1b/publi/affylogin.htm). NF LV clearly showed a lower expression of SLIM1/SLIMMER and of the heart failure marker BNP.

Table 7. Genes differentially expressed in failing LV vs. failing RV

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Description</th>
<th>DCM LV vs. DCM/ICM RV</th>
<th>ICM LV vs. DCM/ICM RV</th>
<th>Functional Category</th>
<th>Molecular Circuit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ratio</td>
<td>SAM</td>
<td>Affy</td>
<td>Ratio</td>
</tr>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z20656</td>
<td>cardiac α-myosin heavy chain</td>
<td>1.08 ↑</td>
<td>+</td>
<td></td>
<td>1.17 ↑</td>
</tr>
<tr>
<td>M83216</td>
<td>aorta caldesmon</td>
<td>1.15 ↑</td>
<td>+</td>
<td></td>
<td>1.88 ↑</td>
</tr>
<tr>
<td>M61906</td>
<td>phosphoinositide-3-kinase, regulatory subunit, 1</td>
<td>1.74 ↑</td>
<td>+</td>
<td></td>
<td>2.00 ↑</td>
</tr>
<tr>
<td>X75346</td>
<td>MAP kinase activated protein kinase</td>
<td>1.39 ↑</td>
<td>+</td>
<td></td>
<td>1.74 ↑</td>
</tr>
<tr>
<td>M61906</td>
<td>phosphoinositide-3-kinase, regulatory subunit, 1</td>
<td>1.32 ↑</td>
<td>+</td>
<td></td>
<td>1.35 ↑</td>
</tr>
<tr>
<td>L75833</td>
<td>BRCA1, Rho7 and vatr genes, and ptp5 gene</td>
<td>1.58 ↑</td>
<td>+</td>
<td></td>
<td>1.53 ↑</td>
</tr>
<tr>
<td>Y17782</td>
<td>heat shock protein B3</td>
<td>-1.26 ↓</td>
<td>+</td>
<td></td>
<td>-1.32 ↓</td>
</tr>
<tr>
<td>U15590</td>
<td>heat shock protein 3</td>
<td>-1.14 ↓</td>
<td>+</td>
<td></td>
<td>-1.10 ↓</td>
</tr>
<tr>
<td>I03191</td>
<td>profilin</td>
<td>-2.35 ↓</td>
<td>+</td>
<td></td>
<td>-2.01 ↓</td>
</tr>
<tr>
<td>L36033</td>
<td>stromal cell-derived factor 1</td>
<td>-2.10 ↓</td>
<td>+</td>
<td></td>
<td>-1.62 ↓</td>
</tr>
<tr>
<td>AL996718</td>
<td>fer-1-like 2, myofilin</td>
<td>-1.84 ↓</td>
<td>+</td>
<td></td>
<td>-1.30 ↓</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z70276</td>
<td>fibroblast growth factor 12</td>
<td>-2.62 ↓</td>
<td>+</td>
<td></td>
<td>NR</td>
</tr>
<tr>
<td>S82297</td>
<td>β2-microglobulin</td>
<td>-1.02 ↓</td>
<td>+</td>
<td></td>
<td>-0.93 ↓</td>
</tr>
<tr>
<td>AE013570</td>
<td>smooth muscle myosin heavy chain SM2</td>
<td>0.72 ↑</td>
<td>+</td>
<td></td>
<td>1.31 ↑</td>
</tr>
<tr>
<td>AF001548</td>
<td>myosin, heavy polypeptide 11, smooth muscle</td>
<td>0.82 ↑</td>
<td>+</td>
<td></td>
<td>1.25 ↑</td>
</tr>
<tr>
<td>HG1111-</td>
<td>Ras-like protein Tc21</td>
<td>0.78</td>
<td></td>
<td></td>
<td>1.73 ↑</td>
</tr>
<tr>
<td>HT1111</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U54617</td>
<td>pyruvate dehydrogenase kinase isofrom 4</td>
<td>0.85 ↑</td>
<td>+</td>
<td></td>
<td>1.98 ↑</td>
</tr>
<tr>
<td>AB020693</td>
<td>reticulin 4</td>
<td>0.81 ↑</td>
<td>+</td>
<td></td>
<td>1.14 ↑</td>
</tr>
<tr>
<td>V01512</td>
<td>cellular oncogene c-fos</td>
<td>-0.08 ↓</td>
<td>+</td>
<td></td>
<td>-1.48 ↓</td>
</tr>
<tr>
<td>J02931</td>
<td>coagulation factor III</td>
<td>-1.00 ↓</td>
<td>+</td>
<td></td>
<td>-1.84 ↓</td>
</tr>
</tbody>
</table>

For details, see legend to Table 4. Functional categories are as listed in Table 3. Up arrows (↑) indicate significantly upregulated genes, and arrows (↓) indicate significantly downregulated genes; ratio values with no arrows were not significantly different.
For example, although it has been suggested, based on animal studies, that apoptosis would be responsible for myocyte loss in aging human hearts, it has now been shown that aging does not influence the percentage of myocyte apoptosis (30) in human cardiac tissue. The same study did show a correlation between gender and apoptosis, but this fact does not affect our study since all samples came from male patients. More generally, we believe that "normal nonfailing" hearts are impossible to obtain. Other researchers have used tissue from donor hearts that could not be transplanted (22, 47). These donor hearts came from individuals that had been declared brain dead. This circumstance in itself or the events leading up to it also might influence gene expression. To address this issue more directly, we compared expression levels of 10 genes in 11 nonfailing hearts to the expression level in our control NF LV. One striking result was that among these nonfailing hearts NF LV displayed the lowest level of BNP expression. BNP is a known marker of heart failure (25), with increasing plasma levels corresponding to the severity of the disease. The lowest expression of BNP in NF LV underlines the "normal aspect" of this heart. Induction of BNP mRNA expression has been shown to occur as rapidly as 4 h after cardiac injury in rats (17). The extremely high expression of BNP in some of the nonfailing heart samples might reflect a deterioration of the hemodynamic status of brain-dead patients occurring shortly before explantation of the heart. The second gene that showed discordance of expression levels in NF LV vs. the additional nonfailing hearts was SLIM1. We found an upregulation of the isoform SLIMMER in failing hearts vs. NF LV using microarrays and an upregulation of both SLIM1 and SLIMMER using real-time PCR. Yang et al. (47) found a downregulation of SLIM1 in failing hearts, whereas Lim et al. (26) found an upregulation of both isoforms in hypertrophied hearts. These different findings may reflect different pathologies, but they may also be the result of the heterogeneity of SLIM1 expression levels in nonfailing hearts. Since for most of the genes tested the expression value of NF LV remained within the gene expression variation of 11 additional nonfailing hearts and since NF LV displayed the lowest level of expression of BNP, we believe that our choice of control tissue is validated. However, we want
to stress that as long as different studies are based on different control samples, different results may be obtained.

**Genes similarly expressed in the four cardiac samples.** We have identified 1,306 genes that are significantly representative of a part of the transcriptional profile of the human heart, based on similar expression levels in all 4 pathophysiological situations. This profile therefore does not contain genes whose expression changes in failing hearts. To obtain a more complete profile, “truly normal” cardiac tissue should be analyzed. In the future maybe this could be obtained from cardiac biopsies, although biopsies are generally performed on patients with cardiac problems. Currently the sensitivity of microarrays is insufficient to analyze the minuscule quantities of cardiac biopsies. Another possibility would be to analyze explanted hearts from nonfailing hearts from individuals with different clinical backgrounds and to identify those genes with similar expression levels in all individuals.

It is generally believed that the expression profile characteristic for a certain tissue consists of few genes that are expressed at a high level (Table 3). Several of those genes, identified in this study, are known to be preferentially expressed in heart and/or skeletal muscle and therefore do not represent housekeeping but cardiac-specific genes. The high level of expression of certain genes involved in metabolism and the large representation of metabolic genes in our total cardiac gene population correlated well with the energy demanding function of the heart. The large group of genes involved in metabolic and sarcomeric proteins. Overall, the distribution of our population of cardiac genes over the different functional categories corresponded well with the literature.

The strongest represented categories (gene and protein expression, metabolism, and cell signaling and communication) were the same, albeit in a different order, as had been found by Yang et al. (47). This group studied gene expression in failing and nonfailing human LV using oligonucleotide microarrays containing ~7,000 genes, and they listed the expression of 473 genes in nonfailing hearts. The in silico analyses of human cardiac cDNA libraries (20, 21) identified more genes involved in cell structure and motility and less in cell signaling and communication. It is possible that this difference is related to the fact that the cDNA libraries had mainly been prepared from whole heart and not just from ventricle, thereby masking cardiac compartment-specific gene expression. The in silico analyses represented the first studies aimed at the description of a catalog of human cardiac genes (5, 21). We present here the first selection of genes representative of part of the human cardiac transcriptome using oligonucleotide microarrays. Currently, transcriptional profiles have also been assessed for human brain (35) and skeletal muscle (6, 7, 34). In the future, similar data will become available for additional normal human tissues. The comparison of the profiles will serve as a guide in the understanding of the functioning of the human body and in the identification of genes that play key roles in the determination of function.

**Differential gene expression.** In the development of heart failure, several molecular circuits have been shown to be involved (12, 19, 23). Factors implicated in the process of cardiac remodeling include the extracellular matrix, oxidative stress, metabolism, calcium signaling, apoptosis, the cytoskeleton, and the sarcomere. Although our approach was limited in the detection of genes involved in heart failure, we did identify genes associated with each of these factors as being differentially expressed in failing heart vs. nonfailing heart.
The downstream structural phase of the remodeling process was represented by changes in expression of genes encoding cytoskeletal proteins, sarcomeric proteins, and extracellular matrix proteins. These changes are responsible for progressive cytoskeletal stiffness, contractile dysfunction, and fibrosis. Some of these changes had already been described, like the downregulation of αMHC in failing LV and RV (8, 28, 31) and the upregulation of α-tubulin in failing LV (18).

We also found additional evidence for a reversal to developmental gene expression, a phenomenon known to occur in cardiac remodeling and heart failure. This evidence consisted of an upregulation of MLC1emb, calponin, and SM22. Some expression changes were indicative of a dedifferentiation process, like the upregulation of other smooth muscle genes (smooth muscle myosin heavy chain and smooth muscle α-actin). Taken together with the data from the literature, our results therefore reinforce the hypothesis that cardiac remodeling involves many structural changes of cardiac tissue.

Structural ventricular remodeling most likely represents the final stage of heart failure. The pathways leading up to this may include disturbed signaling, resulting, for example, in increased apoptosis (33). In our study several apoptosis-related genes were identified as being differentially expressed in failing LV and/or RV, with the one showing the most marked activation being CDKN1A. Apoptosis may be caused by increased oxidative stress (10), a process that marks the transition of hypertrophy to heart failure (39). Stress-inducible metallothionein, which we found to be upregulated in failing LV and RV, is involved in both these processes. This protein functions as an antioxidant, and, in addition, inhibits the production of ANF and its apoptotic effect (24). It has been described that administration of isoprotenerol, a β-receptor agonist, induces cardiac metallothionein levels (32). None of our patients received isoproterenol, and we found no association between administration of other β-receptor agonists (dobutamine, epinephrine) and levels of metallothionein RNA upregulation. Therefore, in our patients the increase of metallothionein is not related to medication.

In addition to genes that are assigned to certain pathways, we also detected aberrant expression of genes of which it is not known in which molecular circuit they are involved. Examples are AF1q, a transmembrane protein (43) also overexpressed in muscle tissue affected by α-sarcoglycan deficiency (11), and SH3BGR, which is preferentially expressed in skeletal muscle and heart (37) and maps to the Down syndrome heart critical region (13).

Relatively few genes (20) were differentially expressed between failing LV and failing RV. Two conclusions could be drawn from these results: 1) LV and RV display similar expression profiles; and 2) LV and RV are similarly affected by heart failure. Genes belonging to the functional category of cell and organism defense (C/OD) were found only to be lower expressed in failing RV vs. failing LV. Most likely this implies that those genes (heat shock protein B3, stromal cell-derived factor 1, and β2-microglobulin) are more severely upregulated by heart failure in LV than in RV. The gene that showed the most marked expression difference between failing LV and RV was profilin, which displayed a more than fourfold lower expression in failing RV compared with failing LV. Profilin is an actin-binding protein that has been implicated in the control of actin polymerization and cytoskeletal reorganization (41, 45, 46). However, questions remain about the exact in vivo function of the protein. Our findings about differential expression of profilin in failing LV and failing RV may lead to novel hypotheses.

Direct comparison of DCM LV and ICM LV showed that, after application of SAM and Affymetrix criteria, no genes were significantly differentially expressed between dilated and ischemic failing hearts. This result underlined the fact that all analyzed tissue came from end-stage failing hearts. The expression profiles that we determined were probably more representative of end-stage heart failure than of DCM or ICM specifically.

Although the analysis of gene expression using (nucleotide) microarrays is a powerful technique, it does have its limitations. Obviously, not all genes are represented (yet) on the array, and therefore, the knowledge that can be acquired from these experiments remains incomplete. In addition, the outcome of microarray analysis is sensitive to the design of the probes on the array. This point was highlighted in our results by the difficulty of discriminating the alternatively spliced isoforms SLIM and SLIMMER. However, with our study we succeeded to add to the clarification of the cardiac transcriptome in different situations. Part of our data had already been described by others and therefore functioned as a confirmation of those findings and a validation of our method. In addition, we presented novel information resulting in an expanded view of human cardiac gene expression. Taken together, our results provide another step forward on the pathway toward improved understanding and treatment of cardiac pathologies.

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