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Right Ventricular Myocardial Biomarkers in Human Heart Failure

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Abstract

Background: Right ventricular (RV) dysfunction contributes to mortality in chronic heart failure (HF). However, the molecular mechanisms of RV failure remain poorly understood, and RV myocardial biomarkers have yet to be developed.

Methods and Results: We performed RNA sequencing (RNA-seq) on 22 explanted human HF RVs and 5 unused donor human heart RVs (DON RV) and compared results to those recently reported from 16 explanted human LVs. We used Bowtie-Tophat for transcript alignment and transcriptome assembly, DESeq for identification of differentially expressed genes (DEGs) and Ingenuity for exploration of gene ontologies. In the HF RV, RNA-seq identified 130,790 total RNA transcripts including 13,272 protein coding genes, 10,831 long non-coding RNA genes and 8,605 pseudogenes. There were 800–1000 DEGs between DON and HF RV comparison groups with differences concentrated in cytoskeletal, basement membrane, extracellular matrix (ECM), inflammatory mediator, hemostasis, membrane transport and transcription factor genes, lncRNAs and pseudogenes. In an unbiased approach, the top 10 DEGs SERPINA3, SERPINA5, LCN6, LCN10, STEAP4, AKR1C1, STAC2, SPARCL1, VSIG4 and F8 exhibited no overlap in read counts between DON and HF RVs, high sensitivities, specificities, predictive values and areas under the receiver operating characteristic curves. STEAP4, SPARCL1 and VSIG4 were differentially expressed between RVs and LVs, supporting their roles as RV-specific myocardial biomarkers.

Conclusions: Unbiased, comprehensive profiling of the RV transcriptome by RNA-seq suggests structural changes and abnormalities in inflammatory processes and yields specific, novel HF RV vs HF LV myocardial biomarkers not previously identified by more limited transcriptome profiling approaches.

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Disclosures
None.

Keywords

Human heart failure; right ventricle; RNA-seq; transcriptome; myocardial; biomarkers

Heart failure (HF) biomarkers have evolved to inform myocardial function and fate and predict clinical outcomes.^{1–3} Biomarkers specifically informing right ventricular (RV) function and fate have yet to evolve.⁴ RV function is an independent predictor of survival in left ventricular (LV) HF, a critical determinant of the outcome of LV mechanical circulatory support, and a more potent predictor of mortality than pulmonary arterial pressure in patients with primary pulmonary hypertensive disorders.⁴ RV-specific biomarkers would therefore be applicable in a wide variety of cardiopulmonary diseases.

Via high-throughput sequencing of fractionated RNA, RNA sequencing (RNA-seq) provides an unbiased, comprehensive profile of polyadenylated [poly(A)] mRNA, including poly(A) noncoding RNA transcripts.^{5,6} We hypothesized that an unbiased RNA-seq approach might yield novel candidate myocardium-specific human HF RV biomarkers. We therefore used RNA-seq to generate the transcriptional profile of end-stage human HF RV myocardium and unused donor RV myocardium to identify candidate RV myocardial biomarkers in an unbiased approach. We then reviewed the transcriptomes of HF RVs with echocardiographic dysfunction versus echocardiographic normal function to identify candidate biomarkers of RV dysfunction in end-stage human HF. Finally, to identify RV-specific biomarkers, we compared our findings in the end-stage HF RV transcriptomes with the RNA-seq findings recently published from a study of end-stage human HF LVs.⁶

Materials and Methods

RV Tissue Acquisition

After Institutional Review Board approval of the study, all subjects before transplantation were enrolled in the Vanderbilt Heart and Vascular Institute Biorepository after signing informed consents. We identified 5 unused donor hearts and 22 end-stage LV HF hearts (11 ischemic, 11 nonischemic) explanted at the time of cardiac transplantation. Immediately after explantation, a small piece of RV tissue free of macroscopic fibrosis or infarction was dissected from a cross-section 5–7 cm above the apex, flash frozen in liquid nitrogen, and stored in –80°C till use.

Clinical Characterization, Including the Definitions of RV Failure and RV Dysfunction

Deidentified clinical data were abstracted for all subjects. We defined RV failure as right atrial pressure >8 mm Hg and cardiac index <2.2 L min^{–1} m^{–2},⁷ recorded at the right heart catheterization performed closest to the time of transplantation. As discussed below, we elected not to analyze the RV RNA-seq results based on the presence or absence of RV failure owing to the small number of subjects who satisfied this hemodynamic definition of RV failure.

The presence or absence of RV dysfunction was determined solely by the 2-dimensional Doppler echocardiogram performed closest to the time of transplantation. The American

Society of Echocardiography (ASE) consensus guidelines for RV echocardiographic function assessment⁸ recommends both a qualitative assessment of RV function based on inspection of all conventional RV imaging planes and performance of 1 specialized RV quantitative functional assessment, such as tricuspid annulus position systolic excursion (TAPSE), RV fractional systolic area change (FAC), or RV index of myocardial performance (RIMP). Each of the recommended individual quantitative echocardiographic measures of RV dysfunction (eg, TAPSE <16 mm, FAC <35%, or RIMP >0.4) is potentially limited by available image acquisition planes, sonographic resolution, pre- and afterload dependency, and earlier cardiac surgery.^{9,10} For example, earlier coronary artery bypass graft surgery (CABG) or aortic valve replacement (history of pericardiotomy or open heart surgery) lowers TAPSE even in the setting of preserved global RV function.¹¹ Given the inherent limitations of each of these quantitative echocardiographic measures of RV function, in accord with the ASE guidelines we used a 2-step method to define echocardiographic RV dysfunction. First, an experienced multimodality coinvestigator (E.B.), blinded to the quantitative echocardiographic measures, hemodynamics, and clinical history, provided a provisional qualitative determination of echocardiographic RV function. Second, this provisional qualitative determination was refined after an unblinded review of the 3 ASE-recommended quantitative echocardiographic measures, TAPSE, FAC, and RIMP. RV dysfunction was then defined as the presence of both (1) dysfunction by qualitative assessment and (2) 2 abnormal quantitative assessments (TAPSE <16 mm, FAC <35%, or RIMP >0.4). Review of the aggregate quantitative RV echocardiographic measures did not result in reclassification of any provisional qualitative RV functional determinations.

RV RNA Preparation

Ninety to 120 mg of tissue per study subject was used for RNA extraction. Total RNA was isolated using RNeasy Mini Kit (Qiagen) following the manufacturer's instructions.

RV RNA-seq

We first performed a quality check of the input total RNA by running an aliquot with the use of the Agilent Bioanalyzer to confirm integrity, and a Qubit RNA fluorometry assay was used to measure concentration. For each library, 100 ng total RNA underwent enrichment for poly(A)-containing mRNA with the use of poly-T oligo-attached magnetic beads.¹² After purification, eluted poly(A) RNA was cleaved into small fragments of 120–210 bp (divalent cations, elevated temperature). The cleaved RNA fragments were copied into 1st-strand cDNA with the use of Superscript II reverse transcriptase and random primers. This was followed by 2nd strand cDNA synthesis with the use of DNA Polymerase I and RNase H. The cDNA fragments then underwent an end-repair process, the addition of a single "A" base, and ligation of the Illumina multiplexing adapters. The products were purified and enriched with the use of polymerase chain reaction to create the final cDNA sequencing library. The cDNA library underwent quality control check with the use of an Agilent Bioanalyzer HS DNA assay to confirm final library size and with the use of an Agilent Mx3005 P qPCR machine using the KAPA Illumina library quantification kit to determine concentration. From a 2-nmol stock, samples were pooled by molarity for multiplexing. From the pool, 12 pmol was loaded into each well for the flow cell with the use of the Illumina cBot for cluster generation. The flow cell was loaded into the Illumina HiSeq 2500

using v3 chemistry and HTA 1.8, and sequenced at paired-end 50 bp with a target of 30 million pass filter reads per library. The raw sequencing reads in BCL format were processed with the use of Casava-1.8.2 for Fastq conversion and demultiplexing. The RTA chastity filter was used and only the pass filter reads were retained for further analysis.

Analysis of RV Transcript Expression

Raw sequence-derived data underwent quality controls with the use of tools such as Fastx Toolkit and Fastqc to identify potential outliers before undergoing any advanced analysis. RNA read alignment and mapping was performed with the use of Bowtie/Tophat, and transcriptome reconstruction was performed with the use of Cufflinks for both mRNA and long noncoding RNA (lncRNA).¹³ For Cufflinks, a minimum reads per kilobase of exon model per million mapped reads (RPKM) value of 1 was required for further analysis.¹² Cufflinks was used also to detect and quantitate alternative spliced transcripts and isoforms.¹⁴

To identify biomarkers discriminating unused donor RVs versus HF RVs, we explored differential gene expression^{15,16} with the use of the negative binomial method DESeq¹⁷ and compared unused donor RVs (n = 5) with 4 HF RV comparison groups: (1) ischemic RV (n = 11), (2) nonischemic RV (n = 11), (3) RV with echocardiographic dysfunction (n = 11), and (4) RV with echocardiographic normal function (n = 11). All tests were adjusted for multiple comparisons and significant at the 2-sided significance level of $P < .05$. Exploratory gene ontology, pathway, and network analysis for differentially expressed genes with RPKM ≥ 1 , adjusted $P < .05$, and log2-fold (L2F) expression ≥ 1.5 was performed with the use of Ingenuity IPA between the unused donor RVs and heart failure RVs. To identify potential candidate biomarkers, we selected the 10 transcripts with the greatest differential expression between unused donor RVs and HF RVs for further analysis of read count ranges and calculation of biomarker sensitivity, specificity, and area under the receiver operating characteristic curve (AUC) with the use of Stata IC13.

To identify biomarkers specific to the RV, we compared the RV versus LV transcript expression for these 10 transcripts. Because we did not perform RNA-seq on our LV samples per our initial study design, we compared our RV RNA-seq transcript expression with recently published LV RNA-seq transcript expression after obtaining permission from the investigators to do so.⁶ We tabulated the RV versus LV RPKM expression differences for these 10 transcripts, where $RPKM = (\text{number of specific transcript reads/kb length of specific transcript}) / (\text{total reads per subject sample} / 1,000,000)$. We then compared the read count distributions between the RV and LV groups for these 10 candidate biomarkers with the use of 2-sample Kolmogorov-Smirnov tests in Stata IC13.

To identify biomarkers discriminating RV with normal echocardiographic function versus RV with echocardiographic dysfunction within the HF RV group, we were cognizant that RNA-seq would identify many transcripts with low (RPKM < 1.0) or absent expression in individual subjects within both groups. As a result, we established the pre hoc criterion that if either group included 1 subjects with a transcript RPKM < 1.0 , then that transcript had to be expressed in all subjects in the other group with RPKM > 1.0 . This criterion was designed to permit identification of biomarkers with either (1) low levels of overall expression (as anticipated, for example, for many lncRNAs) or (2) “physiologic” suppression of expression

in one group but not both groups. We explored the RV echocardiographic normal function versus RV echocardiographic dysfunction biomarkers in 2 ways. First, we compared by inspection the 2,000 top-ranked genes in both groups to identify transcripts meeting the RPKM criteria above most discordantly expressed between groups relative to unused donors. Second, we compared RV echocardiographic normal function versus RV echocardiographic dysfunction group read count transcript expression directly without reference to unused donor transcript expression.

Results

Subjects

The 5 unused donor heart subjects included 3 men and 2 women. The age of the unused donor subjects was 45 ± 6 years (mean $\pm$ SD) and the LV ejection fraction was $60 \pm 10\%$. None of the unused donor subjects had diabetes mellitus, hypertension, or coronary artery disease.

The clinical characteristics of the 22 transplant subjects who underwent RV RNA-seq alone regarding (1) ischemic versus nonischemic etiology of HF and (2) echocardiographic normal RV function versus echocardiographic RV dysfunction are presented in Table 1. In these subjects, the echocardiograms were performed in the course of usual clinical care at a mean 98 ± 85 days (range 1–305 days) before transplantation. Eleven subjects overall had undergone earlier cardiac surgery: 8 subjects, all in the ischemic group, underwent earlier coronary artery bypass grafting, 2 subjects in the nonischemic group underwent earlier mitral valve replacement, and 1 subject in the nonischemic group underwent earlier implantation of an LV assist device. No subject underwent earlier aortic valve replacement or tricuspid valve surgery. Only 4 of the 22 subjects (1 and 3 subjects in the ischemic vs nonischemic groups, respectively, and 2 subjects each in the echocardiographic normal RV and echocardiographic RV dysfunction groups, respectively) had RV failure according to the hemodynamic definition of RV failure used. Because virtually all subjects (1) were treated with continuous intravenous milrinone dosed in part based on serial hemodynamic findings and (2) had frequent, often aggressive, adjustments of diuretic therapy in preparation for transplantation, we considered the catheterization hemodynamic findings to be malleable and likely biased. Exercising caution in interpreting such serial hemodynamic data, we elected not to analyze the RV RNA-seq data according to the presence or absence of RV failure as defined.

Earlier cardiac surgery and intraaortic balloon counterpulsation support before transplantation differed between the ischemic and nonischemic groups. Compared with subjects with echocardiographic normal RV function, subjects with echocardiographic RV dysfunction were more likely to undergo intraaortic balloon counter-pulsation support before transplantation, have significantly larger RV basal dimensions, lower TAPSE, and lower FAC, and have >1 abnormal quantitative assessment of echocardiographic RV function (TAPSE, RIMP, or FAC). There were no significant differences in earlier cardiac surgery or LV ejection fraction between the echocardiographic normal RV function and RV dysfunction groups.

Comparison of the cohort of 16 transplanted subjects from a different transplant center who underwent LV RNA-seq alone with the 22 transplant subjects from our center who underwent RV RNA-seq alone is presented in Table 2. Because clinical data for the LV RNA-seq cohort of 16 patients were obtained at a different transplant center, all data elements were not available for comparison between the 2 cohorts. Available data for comparison demonstrated differences in age, sex, medical therapies, pulmonary arterial systolic pressures, LV end-diastolic dimensions, and the use of ventricular assist device implantation as a bridge to transplantation.

Heart Failure RV Transcriptome

Total Reads and Annotation.—The average number of aligned annotated unadjusted reads per library was 24,732,096 (range 18,607,371–35,024,568). Annotated transcripts in the failing RVs included 13,272 protein-coding genes, 10,866 lncRNAs, and 8,605 pseudogenes (Table 3). As expected, the most abundant RV myocardial transcripts were highly enriched with mitochondrial and sarcomere genes (results not shown), which is consistent with the high metabolic machinery and contractile protein requirements of cardiac muscle and consistent with other quantitative studies of human LV myocardial transcripts.⁶

Isoforms and Alternative Spliced Transcripts.—There were 130,790 total isoforms and alternatively spliced transcripts identified with the use of Cufflinks, and protein-coding genes expressed on average 6 isoforms per gene in HF RVs (Table 3).

Differentially Expressed Genes

Unused Donor RV Versus HF RV Ischemic, Nonischemic, Normal Function, and Dysfunction Comparison Groups.—There were more abundant differentially expressed genes in all unused donor versus HF RV group (ischemic, nonischemic, echocardiographic normal function, echocardiographic dysfunction) comparisons. There were 993 differentially expressed genes in unused donor RV versus nonischemic HF RV, 831 differentially expressed genes in unused donor RV versus ischemic HF RV, 848 differentially expressed genes in the unused donor RV versus HF RV with echocardiographic dysfunction, and 904 differentially expressed genes in unused donor RV versus HF RV with echocardiographic normal function RV.

Results of Ingenuity gene ontology, pathway, and network analyses for unused donor RV versus HF RV comparison groups (ischemic, nonischemic, echocardiographic dysfunction, echocardiographic normal function) were generally similar. Commonly identified networks in HF RVs included “chemotaxis of inflammation, cell-to-cell signaling hematologic system, cell-to-cell signaling growth and development, cardiovascular development and development/tissue morphology, humoral immune response, prothrombin activation,” and “Gαi signaling.” In general, these networks were down-regulated in the HF RVs. For example, Ingenuity predicted decreased inflammation, cell movement, chemotaxis, molecular transport, leukocyte migration, and endothelial cell movement and migration in HF RVs.

RV Myocardial Biomarkers

RV Myocardial Biomarkers Identified in an Unbiased Approach and Comparison With Established HF Biomarkers.—

The aim of the present study was to use RNA-seq to identify potential novel RV myocardial biomarkers in an unbiased approach. Therefore, we used a sum-of-ranks approach across all 4 unused RV versus HF RV group (ischemic, nonischemic, echocardiographic dysfunction, echocardiographic normal function) comparisons to compile the HF RV differentially expressed genes most consistently identified. By means of the sum-of-ranks approach, the top 10 differentially expressed genes so identified were SERPINA3, SERPINA5, LCN6, LCN10, STEAP4, AKR1C1, STAC2, SPARCL1, VSIG4, and F8. These 10 transcripts were all significantly reduced in HF RVs, and the read count ranges demonstrated no overlap with the corresponding unused donor RV read count ranges (Table 4; Fig. 1), supporting their role as candidate RV myocardial biomarkers with high discrimination.

We then compared the RV myocardial read count means and ranges of these potential novel RV myocardial biomarkers with the RV myocardial read count means and ranges of established and/or evolving HF biomarkers.^{1,2} Among the established and/or evolving HF biomarkers (A-type natriuretic peptide [ANP], B-type natriuretic peptide [BNP], troponin I, C-reactive protein, galectin-3 [GAL3], myocardial ST2 [IL1RL1], adrenomedullin [ADM], growth differentiation factor 15, copeptin [arginine vasopressin parent transcript], neutrophil gelatinase-associated lipocalin [LCN2], procalcitonin [calcitonin-related polypeptide alpha parent transcript], neuregulin-1, endothelin-1, and cystatin C [CST3]), ANP (NPPA) transcript read counts were significantly different for all comparison groups and myocardial ST2 and procalcitonin read counts different for some groups. However, the HF RV read count ranges for all established and/or evolving biomarkers overlapped with the unused donor read count ranges, as shown in Figure 1, and exhibited substantial expression variation between HF RVs, as shown in Figure 2.

Compared with these potential novel HF RV biomarkers, established LV HF biomarkers exhibited either (1) high specificity but low sensitivity or (2) low specificity and high sensitivity in discriminating unused donor RV from HF RVs (Fig. 3A and 3B). Because the potential novel and HF RV biomarkers exhibited no read count range overlap between unused donor RVs and heart failure RVs, their sensitivities, specificities, predictive values, and AUCs were all equal to 1.0, as shown for SERPINA3 in Figure 3B. In comparison, each of the established LV HF biomarkers exhibited (1) sensitivity, specificity, and predictive values <1.0, as shown in Figure 3C, and (2) AUC <1.0, as shown in Figure 3B. The AUCs for NPPA, BNP, TNNI3, GAL3, ADM, and CST3 were significantly less than for the potential novel heart failure RV biomarker SERPINA3, as shown in Figure 3B. We explored possible gene ontology and network interrelationships among these 10 potential biomarkers in Ingenuity, as shown in Figures 4 and 5. Ingenuity predicted inhibition of tumor necrosis factor (TNF) and transforming growth factor (TGF) β 1 signaling, activation of fibrin cross-polymer activity, and reduction in activity of multiple effectors of acute phase response signaling.

Myocardial Biomarkers of Echocardiographic Normal RV Versus Echocardiographic RV Dysfunction.—

The top 2,000 genes in the HF RV echocardiographic dysfunction group were ranked by greatest L2F difference relative to unused donors and compared with the L2F values relative to unused donors for the corresponding genes in the HF RV echocardiographic normal function group. This strategy was adopted to identify genes discordantly expressed in the HF RV echocardiographic dysfunction versus HF RV echocardiographic normal function groups while controlling for expression in the unused donor group. Overall, the L2F correlation for these 2,000 genes between HF RV echocardiographic dysfunction and HF RV echocardiographic normal function was high at 0.94. Only 8 of these 2,000 genes exhibited discordant L2F expression values between HF RV echocardiographic dysfunction and HF RV echocardiographic normal function, and only 4 of these 8, RP11–309L24.6 (pseudogene similar to filamin C, gamma, actin-binding protein 280), RP11–395P16.1 (pseudogene HIG1 domain family, member 2A), RP5–857K21.10 (pseudogene steroid hydrogenase), and CYP1A1 (cytochrome P450, family 1, subfamily A) exhibited significant differential expression according to 2-sample Kolmogorov-Smirnov testing (Table 5).

Next, using a similar approach and strategy, the top 2,000 genes in the echocardiographic normal function group were ranked by greatest L2F difference relative to unused donors and compared with the L2F values relative to unused donors for the corresponding genes in the echocardiographic dysfunctional group. Overall the correlation for these 2,000 genes was high at 0.95. Only 5 of the 2,000 genes exhibited discordant L2F between the echocardiographic normal function versus echocardiographic dysfunction groups, and of these only 1 gene, CYP1A1, exhibited significant differential expression according to 2-sample Kolmogorov-Smirnov testing (Table 5). By this approach, CYP1A1 thus was identified as a possible biomarker in distinguishing HF RVs with echocardiographic normal function versus echocardiographic dysfunction.

In a different approach, we then examined all 904 unused donor versus echocardiographic normal transcripts and all 848 unused donor versus echocardiographic dysfunction transcripts with adjusted $P < .05$ with the use of DESeq and eliminated all transcripts with $P < .05$ in both unused donor versus echocardiographic normal and unused donor versus echocardiographic dysfunction groups to identify discordant transcripts relative to unused donors in either group. This eliminated 594 out of 904 (65.7%) echocardiographic normal function transcripts and 594 out of 848 (71.1%) echocardiographic dysfunction transcripts. We then compared the echocardiographic normal function and echocardiographic dysfunction read count ranges directly for all the remaining 564 (310 echocardiographic normal, 254 echocardiographic dysfunction) transcripts by means of 2-sample Kolmogorov-Smirnov testing. Out of these 564 transcripts, only 7 transcripts exhibited $P < .05$ between the echocardiographic normal and echocardiographic dysfunction groups: CHST7 (carbohydrate N-acetylglucosamine 6-O sulfurtransferase), ENSG00000259366.1 (novel transcript, sense intronic to HMBOX1), ENSG00000268157.1 (novel transcript, antisense to TEAD2), FHAD1 (forkhead-associated phosphopeptide-binding domain 1), IL1RL1 (interleukin-1 receptor-like 1, ST2), RP11–320N7.3 (heat shock 70kDA protein 8 pseudogene 5), and VIPR1 (vasoactive intestinal peptide receptor 1; Table 6). CYP1A1 was not included in

these 7 transcripts. Because each of these 7 transcripts exhibited overlap in read count range between echocardiographic normal and echocardiographic dysfunction groups, none of them perfectly discriminated between echocardiographic normal and echocardiographic dysfunction groups (Table 6).

Heart Failure RV Versus Heart Failure LV Biomarkers.—To gain insight into possible differential HF LV versus HF RV expression patterns overall, we correlated the L2F gene expression values for the top 25 up-regulated and top 25 down-regulated HF LV mRNA genes from the recently published HF LV RNA-seq study⁶ to the corresponding gene expression L2F values from the present study HF RV RNA-seq group. The L2F value correlation between the heart failure LV versus heart failure RV top 25 up-regulated mRNA transcripts was modest at 0.34, and the L2F value correlation between the HF LV versus HF RV top 25 down-regulated mRNA transcripts was more modest at 0.20.

Because the recent HF LV RNA-seq study did not include echocardiographic normal function versus echocardiographic dysfunction groups (ie, all HF LVs had echocardiographic dysfunction by definition), we did not compare echocardiographic normal versus echocardiographic dysfunction groups for HF LVs. We did compare the HF RV RPKMs and read counts for the top 10 RV biomarker genes in the present study with corresponding HF LV RPKMs and gene read counts reported in the recent HF LV RNA-seq study. According to 2-sample Kolmogorov-Smirnov testing, STEAP4 ($P = .02$), SPARCL1 ($P = .001$), and VSIG4 ($P = .005$) were differentially expressed in HF RV versus HF LV (Table 7), supporting their role as HF RV-specific biomarkers.

Discussion

In this study, we used RNA-seq deep sequencing to profile the RV poly(A) RNA transcriptome in comprehensive and unbiased fashion. RNA-seq deep sequencing allowed a view of the rich complexity of the RV transcriptome and the identification of potential novel RV myocardial biomarkers not previously identified by more limited transcriptional profiling approaches.

Qualitative Transcript Expression

As anticipated, we found abundant expression of both protein-coding and non-protein-coding poly(A) mRNAs with particularly high abundance of mitochondrial and structural protein gene transcripts.^{18–22} The high abundance expression of selected pseudogenes,²³ lncRNAs,^{24,25} and isoforms²⁶ was also striking.

Differentially Expressed Genes

We found relatively few differentially expressed genes between the ischemic versus nonischemic HF RVs. We found a much larger number of differentially expressed genes (800–1,000 genes) between the unused donor versus HF RV ischemic, nonischemic, echocardiographic dysfunction, and echocardiographic normal function comparison groups. These differentially expressed genes tended to be relatively conserved across all the various HF RV comparison groups. By a sum of ranks approach, 10 transcripts were consistently

reduced among the top 25 differentially expressed genes in all HF RV comparison group, including serine protease inhibitors SERPINA3 and SERPINA5, hydrophobic ligand transporters LCN6 and LCN10, reductases AKR1C1 and STEAP4, ion-binding protein STAC2, secreted extracellular matrix components protein SPARCL1, coagulation factor F8, and lymphokine VSIG4, as more fully described in Table 8. These 10 transcripts were selected as potential novel RV myocardial biomarkers.

Signaling, Metabolic, Structural, and Growth Factor Gene Families and Comparison With Earlier Microarray Transcriptome Studies

Few human LV or RV RNA-seq studies have been published to date.⁶ Although it was not our primary focus, we sought to compare qualitatively our aggregate findings with earlier micro-array human LV HF transcriptome studies.²⁷ Our findings suggest more prominent transcriptional changes in selected myocardial structural components (microtubules, extracellular matrices, basement membranes), selected membrane and cell-to-cell signaling systems (eg, TGF β and ERBB), and inflammatory signaling systems than in previously described neurohormonal, calcium signaling, and kinase systems. Exploratory gene ontology, pathways, and networks analyses with the use of Ingenuity confirmed these findings, highlighting the importance of down-regulated transmembrane peptide and glycoprotein signaling, extracellular matrix signaling, cytokine signaling, hematopoietic lineage signaling, inflammation, and wound healing in the HF RV. Taken together, these results sharpen the focus on the roles of cell-to-cell transmembrane communication, basement membrane composition and signaling, extracellular matrix composition and signaling, inflammation, and hemostasis/hematopoietic-associated factors in the HF RV. The regulatory roles of the differentially expressed lncRNAs and pseudogenes also warrant additional study (eg, as possible competing endogenous RNAs in the regulation of mRNA and microRNA).

RV Myocardial Biomarkers

Reliable myocardial biomarkers informing RV function and fate have not been identified. Such myocardial biomarkers would be applicable to a variety of common challenging clinical contexts encountered in managing LV HF, eg, the timing and appropriateness of RV mechanical circulatory support following LV mechanical circulatory support. All established and/or evolving HF biomarkers, however, showed overlap in the myocardial read count ranges between HF RVs and unused donor RVs, potentially limiting their precision as myocardial biomarkers. In an unbiased RNA-seq approach, the transcripts with the lowest sum of ranks across all unused donor versus the HF ischemic, nonischemic, echocardiographic dysfunction, and echocardiographic normal function RV comparison groups—SERPINA3, SERPINA5, LCN6, LCN10, STEAP4, AKR1C1, STAC2, SPARCL1, VSIG4, and F8—exhibited sharp reductions in expression, nonoverlapping read count ranges, high sensitivities, specificities, predictive value, and AUCs, supporting their candidacy as RV myocardial biomarkers.

These 10 potential RV myocardial biomarker proteins have diverse functions in signaling, transport, metabolism, extracellular matrix remodeling, immune response, and hemostasis as annotated by the online gene ontology search site Genecards (accessed at

www.genecards.org with embedded links to the UniprotKB/Swissprot database; Table 7). Interestingly, all 10 of these transcripts were down-regulated in the HF RVs. Exploratory gene ontology and network analysis with these 10 biomarkers supported their roles in inhibition of wound healing (eg, TNF and TGF- β 1 inhibition), promotion of hemostasis (eg, activation of fibrin cross-polymer), and inhibition of acute phase response signaling (diverse pathways and effectors).

Our strategy in searching for RV myocardial biomarkers to discriminate echocardiographic normal function versus echocardiographic dysfunction was to control for the “background” expression of genes in the unused donor hearts, and therefore we anticipated a set of biomarkers different from those identified in the initial unbiased unused donors versus pooled HF RV approach. We identified by means of the different L2F, and our adjusted *P* value approaches used several candidate echocardiographic normal function versus echocardiographic dysfunction biomarkers, including CYP1A1, CHST7, ENSG00000259366.1, ENSG00000268157.1, FHAD1, IL1RL1, RP11-320N7.3, and VIPR1. However, each of these biomarkers exhibited overlap in read count range between the echocardiographic normal function and echocardiographic dysfunction groups and were therefore less discriminatory than the 10 biomarkers identified in the initial unused donor versus pooled HF RV unbiased approach. There are several reasons why discriminatory HF RV echocardiographic normal function versus HF RV echocardiographic dysfunction biomarkers failed to emerge more clearly. First, the echocardiographic assessment of RV function is inherently less precise than the echocardiographic assessment of LV function (owing to the constraints of RV 3-dimensional geometry reconstruction and the greater RV load dependence), and reliance on echocardiography alone may not have provided an accurate reflection of intrinsic RV function. Second, echocardiography was performed in the course of usual care of the subjects while awaiting transplantation, and therefore the duration of time from the performance of the echocardiogram to explantation was variable (98 ± 85 days, range 1–305 days). Third, the “common milieu” of end-stage LV HF present at the time of explantation may have effected RV gene expression independently from RV echocardiographic function.

We performed RNA-seq only on RV myocardium and not on the paired end-stage LV myocardium of our subjects. Our collaborators performed RNA-seq only on end-stage LV myocardium and not on the paired RV myocardium of their subjects.⁶ To our knowledge, our collaborators’ study is the only study of end-stage HF LV RNA-seq published to date.⁶ Therefore, we pooled our RV RNA-seq results with our collaborators’ previously published LV RNA-seq results to explore RV versus LV biomarker expression differences. With the use of this approach, we found that 3 genes of the 10 candidate biomarker genes identified in the unbiased unused donor RV versus HF RV approach—STEAP4, SPARCL1, and VSIG4—were significantly down-regulated in RV versus LV end-stage human myocardium. This selective down-regulation in the RV supports their candidate roles as RV-specific biomarkers in end-stage human HF. Further studies are warranted to validate these RV-specific candidate biomarkers.

Study Limitations

This study was limited to end-stage human myocardium. By design, we analyzed only RV tissue from subjects at our center. All our subjects were treated with medications, including inotropes, before transplantation that likely altered gene expression in the RV. RNA-seq has inherent limitations owing to 5' bias and dependence on library preparation methodology.¹² As demonstrated, substantial biologic variation was found in read counts between subjects. The pooling of samples with such read count variation challenges statistical inference based on an assumed distribution of read counts. Statistical significance as in all such studies is accepted as a “proxy” for biologic significance, realizing that meaningful biologic variation may or may not result in statistical differences in expression if there are threshold, graduated, or nonlinear expression effects on translation. We compared RVs from subjects with end-stage LV failure with the RVs of unused human donors. Unused human donors do not have “normal” RV myocardium owing to the state of brain death before harvest. Earlier studies have reported considerable heterogeneity in gene expression of unused donors due to clinical factors and harvest/storage factors.¹⁸ We compared our RV RNA-seq findings in our cohort of 22 transplanted subjects with the LV RNA-seq findings from a cohort of 16 transplant subjects from another center.⁶ The clinical differences between the 2 separate RV and LV cohorts likely arose from biases originating from differential study design and clinical practice differences at different transplant centers. For example, the LV RNA-seq cohort from the different transplant center was composed exclusively of patients who underwent implantation of an LV assist device (VAD) as a bridge to transplantation to ascertain LV transcriptional responses before and after VAD implantation.⁶

We compared the RV RNA-seq findings between subjects with RV normal function and RV dysfunction as defined solely by echocardiography. As discussed above, earlier cardiac surgery may have influenced the prevalence of echocardiographic RV dysfunction as defined by quantitative echocardiographic measurements. We did not compare the RV RNA-seq findings between subjects with RV failure as defined by the hemodynamic findings at right heart catheterization for 2 reasons. First, because only 4 of our 22 subjects had RV failure as so defined, comparative analysis of such a small number of subjects would enhance the likelihood of detecting chance associations. Second, because virtually all subjects were treated with varying doses of intravenous inotropes, vasodilators, and diuretics before transplantation which might confound the hemodynamic definition of RV failure used, we exercised great caution in interpreting the hemodynamic results. The variable timing of the echocardiograms and right heart catheterizations relative to transplantation is an unavoidable and acknowledged limitation of this study.

Finally, although rates of mRNA transcription rather than degradation appear to be most important in determining mRNA levels, degradation rates and mRNA maturation vary significantly among genes,²⁸ and contribute to differences in mRNA levels under certain conditions and in different cells.²⁹ In homogenizing tissue, we could not uncover the known fold differences in total RNA content known to exist between different cells due to global transcriptional amplification or repression.²⁹ Finally, all such studies are inherently limited by the lack of understanding of fundamental aspects of gene expression, including the relationship of transcribed mRNA to translated protein. In a recent study in mouse

fibroblasts, for example, proteins (1) had a median half-life of 46 hours compared with 9 hours for mRNA, (2) were on average 2,800 times more abundant than their mRNAs, and (3) had levels that imperfectly correlated with their mRNA levels ($R^2 = 0.41$, log-log scale). Thus, extrapolation from mRNA transcription levels to protein translation and integrated biologic processes is necessarily imperfect.³⁰

Conclusion

Compared with unused human donors, substantial differences in protein-coding, pseudogene, and lncRNA gene expression occur in HF RVs. Compared with unused donor RVs, HF RVs are marked by differential biologic processes, including cell-to-cell signaling, inflammation, wound response, hemostasis, and extracellular matrix signaling. In an unbiased approach, RNA-seq identifies potentially novel candidate HF RV myocardial biomarkers. Further studies are warranted to validate the candidate RV myocardial biomarkers discriminating (1) HF RV myocardium from unused donor RV myocardium, (2) HF RV myocardium with echocardiographic dysfunction from HF RV myocardium with echocardiographic normal function, and (3) HF RV myocardium from HF LV myocardium identified in this study.

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Fig. 1.

Read count ranges for selected biomarkers. The lowest and highest read counts in the donor (DON) right ventricles (RVs; $n = 5$) vs heart failure (HF) RVs ($n = 22$) are presented for SERPINA3, SERPINA6, LCN6, LCN10, STEAP4, ANP, BNP, Gal3, ST2, and Cystatin C. Top row: for these 5 established biomarkers, there was no overlap in RV myocardial read ranges. Bottom row: for these 5 established biomarkers, there was overlap in RV myocardial read ranges.

Fig. 2.

Read counts for NPPA, NNPB, LGALS3, ST2, and CST3. The histograms show adjusted counts for NPPA, NNPB, GAL3, ST2, and CST3 for all 22 heart failure patients. There were substantial differences in counts for all 5 different transcripts between heart failure right ventricles. As discussed in the text, neither clinical characteristics nor NPPA promoter-associated transcription factors accounted for the variation in NPPA read counts between subjects.

Fig. 3.

Panel A displays the sensitivity and specificity for established heart failure biomarkers. Panel B displays the area under the received operating characteristic (ROC) curve (AUC) performance for established heart failure biomarkers compared to SERPINA3 AUC. Panel C displays the sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively) and ROC areas for established heart failure biomarkers with the cut-off values as shown. * $P < .05$ testing for difference from SERPINA3 AUC. PPV, positive predictive value; NPV, negative predictive value.

Fig. 4. Ingenuity “Cardiovascular Disease, Organismal Injury, and Abnormalities” network results and predictions for potential novel right ventricular biomarkers. Given the observed down-regulation of SERPINA3, SERPINA5, F8, VSIG4, SPARCL1, STEAP4, and AKRIC1 (*green*; see inset Prediction Legend), Ingenuity predicts inhibition of TNF, TGF- β 1, APP, CTSG, SYP, KLK6, chymotrypsin, F2, F11, NADPH oxidase, and AChR (*blue*) and activation of CTF1, serine protease, allopregnanolone, and FIRSP12 (*brown*). (Color version of the figure is available online.)

Fig. 5.

Ingenuity “Coagulation System” and “Acute Phase Response Signaling” canonical pathway effects identified from novel right ventricular biomarkers as inputs. (A) Ingenuity “Coagulation System” canonical pathway. Given the observed down-regulation of SERPINA5 and F8, Ingenuity predicts activation of the F3/F5a/F7a/F10a complex, F2a, Protein C, Fibrin, F13a, and Fibrin cross-link polymer (*brown*). (B) Ingenuity “Acute Phase Response Signaling” canonical pathway. Given the observed down-regulation of SERPINA5 and F8, Ingenuity predicts widespread inhibition of a variety of acute phase signaling effectors, including STAT3, NF- κ B, c-JUN, NF-IL6, glucocorticoid receptors, and corticosteroid receptors (*blue*). (Color version of the figure is available online.)

Clinical and Echocardiographic Characteristics According to Heart Failure Etiology and Echocardiographic (Echo) Right Ventricular (RV) Function

Characteristic	Nonischemic HF Etiology	Ischemic HF Etiology	Echo Normal RV Function	Echo RV Dysfunction
No. of subjects	11	11	11	11
Clinical				
Age, y (mean $\pm$ SD)	49 $\pm$ 14	54 $\pm$ 9	53 $\pm$ 10	50 $\pm$ 13
Sex, n (M/F)	7/4	9/2	8	8
Diabetes, n	5	8	7	6
Serum creatinine, mg/dL (mean $\pm$ SD)	1.6 $\pm$ 0.7	1.6 $\pm$ 0.7	1.7 $\pm$ 0.7	1.5 $\pm$ 0.8
Previous cardiac surgery, n	3	8*	4	7
Hematocrit, % (mean $\pm$ SD)	34 $\pm$ 5	35 $\pm$ 6	35 $\pm$ 6	34 $\pm$ 6
Therapy, n				
ACEI/ARB	7	7	7	7
Beta-blocker	10	9	11	8
Aldosterone inhibitor	9	9	9	9
PDE V inhibitor	4	1	3	2
Inotropic therapy	11	10	11	10
Intra-aortic balloon pump	0	5*	0	5 [†]
Ventricular assist device	1	0	0	1
Hemodynamics (mean $\pm$ SD)				
Right atrial pressure, mm Hg				
Pulmonary artery pressure, mm Hg	26 $\pm$ 8	27 $\pm$ 10	30 $\pm$ 9	24 $\pm$ 8
Pulmonary capillary wedge, mm Hg	16 $\pm$ 8	17 $\pm$ 7	19 $\pm$ 7	16 $\pm$ 8
Pulmonary vascular resistance, Woods units	2.2 $\pm$ 0.6	2.6 $\pm$ 1.7	2.7 $\pm$ 1.5	2.3 $\pm$ 0.9
Cardiac index, L min ⁻¹ m ⁻²	2.2 $\pm$ 0.4	2.1 $\pm$ 0.4	2.4 $\pm$ 0.5	2.0 $\pm$ 0.2
RV failure (RAP > 8 mm Hg, CI < 2.2 L min ⁻¹ m ⁻²), n	3	1	2	2
Echocardiography (mean $\pm$ SD) RV basal, cm	4.6 $\pm$ 0.5	4.5 $\pm$ 0.8	4.2 $\pm$ 0.5	4.8 $\pm$ 0.6 [†]
RV long axis, cm	8.3 $\pm$ 1.1	7.3 $\pm$ 0.6	7.4 $\pm$ 0.9	8.0 $\pm$ 0.8
TAPSE, mm	14.3 $\pm$ 4.9	11.7 $\pm$ 3.9	16.5 $\pm$ 2.8	10.8 $\pm$ 3.1 [†]
RIMP	54.7 $\pm$ 25.6	49.6 $\pm$ 10.7	43.1 $\pm$ 16.3	53.9 $\pm$ 21.7

Characteristic	Nonischemic HF Etiology	Ischemic HF Etiology	Echo Normal RV Function	Echo RV Dysfunction
FAC, %	29.2 ± 20.7	33.2 ± 11.4	40.4 ± 16.3	24.7 ± 14.2 [‡]
RAP (estimated), mm Hg	8.7 ± 4.7	6.3 ± 5.1	7 ± 4	7 ± 5
Moderate or severe dysfunction, n	5	6	0	5 [‡]
Abnormal TAPSE, RIMP, or FAC per subject, n	—	—	1.0 ± 0.8	2.7 ± 0.5 [‡]

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; PDE V, phosphodiesterase type V; RAP, right atrial pressure; CI, cardiac index; TAPSE, tricuspid annular plane systolic excursion; RIMP, right ventricular index of myocardial performance; FAC, right ventricular fractional area change.

* $P < .05$: comparison of nonischemic HF etiology with ischemic HF etiology.

[‡] $P < .05$: comparison of echocardiographic normal RV function with echocardiographic RV dysfunction.

Table 2.

Clinical and Echocardiographic Characteristics of the Separate RV and LV Study Cohorts

Characteristic	RV Cohort	LV Cohort
Subjects	22	16
Clinical		
Age, y *	51 ± 11	58 ± 4
Sex (male), n *	16	13
Diabetes, n	13	7
Prior cardiac surgery, n	11	7
Serum creatinine, mg/dL	1.6 ± 0.8	N/A
Hematocrit, %	35 ± 6	N/A
Etiology		
Ischemic, n	11	8
Therapy, n		
ACEI/ARB *	14	3
Beta-blocker *	19	12
Aldosterone inhibitor	18	N/A
PDE V inhibitor	5	N/A
Inotropic therapy *	21	5
Intra-aortic balloon pump	5	N/A
Ventricular assist device *	1	16
Hemodynamics (mean ± SD)		
Right atrial pressure, mm Hg	9 ± 6	N/A
Mean pulmonary artery systolic pressure, mm Hg	39 ± 13	49 ± 14 *
Mean pulmonary artery diastolic pressure, mm Hg	17 ± 8	23 ± 9
Mean pulmonary artery pressure, mm Hg	27 ± 9	N/A
Pulmonary capillary wedge, mm Hg	18 ± 8	N/A
Pulmonary vascular resistance, Woods units	2.5 ± 1.2	N/A
Cardiac index, L min ⁻¹ m ⁻²	2.2 ± 0.4	N/A
Echocardiography (mean ± SD)		
Left ventricular end-diastolic dimension, mm	61.4 ± 10.0	71.0 ± 7.0 *
Left ventricular ejection fraction, %	20.7 ± 9.9	16.0 ± 4.9

Abbreviations as in Table 1.

* $P < .05$: comparison of RV and LV cohorts.

Table 3.

Cumulative Number of Genes and Transcripts Expressed* Compared with Gencode

	Total in Master (GRCCh37_63; gencode.v16)	Expressed in Normal RV (n = 5)	Expressed in Normal LV (n = 5)	Expressed in Failing RV (n = 22)
Protein-coding genes	20,007	12,492	12,320	13,272
Long noncoding RNA genes	13,220	10,732	10,719	10,831
Pseudogenes (processed and unprocessed)	13,196	8,486	8,462	8,605
Transcripts				
Total no. of transcripts	174,930	125,722	124,283	130,790
Protein-coding transcripts (full and partial length)	125,376	88,521	87,493	93,058
Long noncoding RNA loci transcripts	22,444	17,488	17,450	17,743

RV, right ventricle; LV, left ventricle.

* For this study, a gene was considered to be expressed if the fragments per kilobase of gene model per million mapped reads were 1.

Table 4.

Differentially Expressed Genes: Possible Myocardial Biomarkers of RV Failure

Gene	Donor Hearts (Unadjusted Read Counts, Mean $\pm$ SD)	Donor Hearts (Unadjusted Read Counts, Range)	HF RVs (Unadjusted Read Counts, Mean $\pm$ SD)	HF RVs (Unadjusted Read Counts, Range)
Potential novel RV biomarkers				
SERPINA3 [*]	691 $\pm$ 631	1,089–2,737	126 $\pm$ 120	12–429
SERPINA5 [*]	183 $\pm$ 56	96–242	17 $\pm$ 9	7–41
LCN6 [*]	406 $\pm$ 694	433–2,194	91 $\pm$ 63	20–186
LCN10 [*]	948 $\pm$ 478	227–1,538	40 $\pm$ 30	3–108
STEAP4 [*]	1,562 $\pm$ 413	1,146–2,129	373 $\pm$ 121	151–576
AKR1C1 [*]	1,471 $\pm$ 890	442–2,662	219 $\pm$ 74	92–398
STAC2 [*]	67 $\pm$ 39	29–148	5 $\pm$ 3	1–12
SPARCL1 [*]	22,051 $\pm$ 9,662	13,100–35,248	6,098 $\pm$ 1,850	3,439–9,008
VSIG4 [*]	488 $\pm$ 274	271–963	60 $\pm$ 25	14–112
F8 [*]	2,747 $\pm$ 555	1,850–3,363	775 $\pm$ 216	437–1,291
Established LV biomarkers				
ANP [†]	1,583 $\pm$ 1,858	105–4128	42,373 $\pm$ 59,245	628–228,685
BNP	9,777 $\pm$ 20,731	330–46,859	18,150 $\pm$ 25,683	171–101,126
TNNI3	149,014 $\pm$ 73,644	89,249–272,223	299,358 $\pm$ 126,726	112,622–717,437
CRP	0.6 $\pm$ 0.5	0–1	0.4 $\pm$ 0.6	0–1
Galectin-3 (LGALS3)	4,759 $\pm$ 567	4,224–5,443	4,222 $\pm$ 835	2,453–5,292
ST2 (IL1RL1) [‡]	503 $\pm$ 669	54–1,688	52 $\pm$ 71	2–277
IL-33	491 $\pm$ 208	229–753	251 $\pm$ 121	78–473
Adrenomedullin	277 $\pm$ 51	225–346	241 $\pm$ 133	126–666
GDF-15	6 $\pm$ 3	2–11	7 $\pm$ 8	0–33
Copeptin (AVP)	2 $\pm$ 2	0–5	0.4 $\pm$ 0.6	0–2
NGAL (LCN2)	2 $\pm$ 3	0–7	2 $\pm$ 2	0–6
Procalcitonin [‡]	7 $\pm$ 9	0–22	1 $\pm$ 1	0–3
Cystatin C	8757 $\pm$ 1,512	6,368–10,166	6,677 $\pm$ 2,071	4,006–11,516

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Gene	Donor Hearts (Unadjusted Read Counts, Mean ± SD)	Donor Hearts (Unadjusted Read Counts, Range)	HF RV's (Unadjusted Read Counts, Mean ± SD)	HF RV's (Unadjusted Read Counts, Range)
Endothelin-1	130 ± 77	62–234	49 ± 22	19–90
Neuregulin-1	2 ± 3	0–6	4 ± 5	0–15

* Significantly different between all failing RV comparison groups without any overlap in the unadjusted read counts within the unused donors.

[†] Significantly different between unused donors and all failing RV comparison groups but with overlap in the unadjusted read counts within the unused donors.

[‡] Significantly different between unused donors and some failing RV comparison groups but with overlap in unadjusted read counts within unused donors.

Table 5.

Genes With Discordant Log2-Fold (L2F) Expression Relative to Unused Donors in the Echocardiographic (Echo) Normal Function Versus Echocardiographic Dysfunction Groups

	Echo Normal Function Group L2F Expression Relative to unused Donor Group	Echo Normal Function vs Unused Donor (<i>P</i> Value by DESeq)	Echo Dysfunction Group L2F Expression Relative to Unused Donor Group	Echo Dysfunction Group vs Unused Donor (<i>P</i> Value by DESeq)
Genes ranked by echo dysfunction L2F				
RP11-309L24.6 *	-0.29	1.0	3.36	1.18E-7
RP11-395P16.1 *	-1.72	1.0	4.52	.0091
RP5-857K21.10 *	-0.21	1.0	2.27	.0029
CYP1A1 *	-0.21	0.23	3.43	.0035
ARC *	-0.05	1.0	1.94	.056
snoU13 *	-3.87	0.11	-1.99	.064
MF12 *	-0.20	1.0	0.90	.094
ENSG00000232079.1 *	-0.13	1.0	2.02	.14
Genes ranked by echocardiographic normal function L2F				
snoU13 †	3.87	0.11	-1.99	.064
ITLN1 †	1.63	0.16	-0.07	1.0
RNF17 †	-2.84	0.17	0.14	1.0
CENPF †	-1.27	0.17	0.12	1.0
CYP1A1 †	-1.55	0.23	3.43	0.0035

* Identified by review of L2F values of the top 2,000 genes ranked by L2F in the dysfunction group.

† Identified by review of L2F values of the top 2,000 genes ranked by L2F in the normal function group.

Table 6.

Genes Differentially Expressed in the Echocardiographic Normal Function Versus Echocardiographic Dysfunction Groups: Potential Biomarkers

Gene	Echocardiographic Normal Function				Echocardiographic Dysfunction				P Value*
	Read Count Mean	Read Count SD	Read Count Min	Read Count Max	Read Count Mean	Read Count SD	Read Count Min	Read Count Max	
CHST7	143.4	30.9	108	220	259.4	127.9	140	544	.021
ENSG00000259366.1	5.6	2.9	2	11	2.7	2.8	0	10	.01
ENSG00000268157.1	5.5	2.1	1	7	2.7	2.6	0	9	.01
FHAD1	13.7	7.7	2	27	6.1	5.2	1	16	.01
IL1RL1	20.2	16.1	2	58	84.6	89.5	2	277	.036
RP11-320N7.3	4.6	5.1	0	17	14.0	8.9	0	30	.004
VIPR1	82.8	36.9	39	173	47.9	22.7	21	88	.036

* 2-sample Kolmogorov-Smirnov testing.

Table 7.

Heart Failure RV RPKMs Versus Heart Failure LV RPKMs for “Top 10” RV Biomarkers Identified With the Use of an Unbiased Approach

Gene	RV RPKM	LV RPKM	<i>P</i> Value*
SERPINA3	2.74 ± 3.37	6.33 ± 5.65	.056
SERPINA5	0.37 ± 0.27	0.24 ± 0.22	.248
LCN6	1.51 ± 1.07	0.70 ± 0.31	.1
LCN10	2.42 ± 2.23	1.20 ± 0.46	.128
STEAP4	3.96 ± 1.37	7.44 ± 3.28	.02
AKR1C1	5.66 ± 2.06	9.71 ± 9.13	.683
STAC2	0.070 ± 0.047	0.04 ± 0.03	.419
SPARCL1	111.24 ± 29.61	231.14 ± 61.38	.001
VSIG4	1.35 ± 0.71	6.89 ± 4.28	.005
F8	10.55 ± 2.85	11.85 ± 2.57	.248

*
2-sample Kolmogorov-Smirnov testing.

Table 8.

Potential RV Biomarkers: Genes, Proteins, Functions, GO Annotation Terms

Gene	Encoded Protein	Function	GO Annotation Terms
SERPINA3	Serpin peptidase inhibitor, clade A member 3	Plasma protease inhibitor, serine protease inhibitor class; superpathways include Jak/Stat pathway and interleukin-6 signaling; although physiologic function is unclear, it can inhibit neurophil cathepsin G and mast cell chymase, both of which can convert angiotensin-1 to the active angiotensin-2	Serine-type endopeptidase inhibitor activity; DNA binding; Protein binding
SERPINA5	Serpin peptidase inhibitor, clade A member 5	Heparin-dependent serine protease inhibitor that can inhibit several serine proteases; plays diverse roles in hemostasis and thrombosis in multiple organs; acts as a procoagulant and proinflammatory factor by inhibiting the anticoagulant activated protein C factor as well as the generation of activated protein C factor by the thrombin/thrombomodulin complex; acts as an anticoagulant factor by inhibiting blood coagulation factors like prothrombin, factor XI, factor Xa, plasma kallikrein and fibrinolytic enzymes such as tPA and uPA	Heparin binding; Protease binding; Glycosaminoglycan binding; Serine-type endopeptidase activity; Extracellular space; External side of plasma membrane
LCN6	Lipocalin 6	Hydrophobic ligand-binding transport protein; common structure (8-stranded antiparallel b-barrel, cup-shaped ligand-binding pocket or calyx); lipocalins generally bind small hydrophobic ligands and transport them to specific cells.	Extracellular region
LCN10	Lipocalin 10	See above for lipocalin 6; may act as a retinoid carrier protein within the epididymis (heart function unknown)	Extracellular region
STEAP4	Six transmembrane epithelial antigen of prostate family member 4	Metalloreductase residing in the Golgi apparatus; reduces both Fe ³⁺ to Fe ²⁺ and Cu ²⁺ to Cu ⁺ , using NAD ⁺ as acceptor; may be involved in adipocyte development and metabolism and associated with obesity and insulin-resistance; plays a role in systemic metabolic homeostasis, integrating inflammatory and metabolic responses; regulates inflammatory cytokines and inhibits anchorage-independent cell proliferation	Oxidoreductase activity; Nucleotide binding; Phosphogluconate dehydrogenase (decarboxylating) activity
AKR1C1	Aldo-keto reductase family 1, member C1	Aldo/keto reductase which catalyzes the reaction of progesterone to the inactive form 20-alpha-hydroxy-progesterone using NADH and/or NADPH as cofactors	Oxidoreductase activity, acting on NAD(P)H; Aldo-keto reductase (NADP) activity.
STAC2	SH3 and cysteine-rich domain 2	Contains SH3 and cysteine-rich and zinc-finger domains; binds Zn ²⁺ ; associates with a variety of adenylate cyclase enzymes and Smad proteins; involved in signal transduction (best described to date in neural tissue)	Metal ion binding; Protein binding; Intracellular signal transduction
SPARC1	SPARC-like 1 (hevin)	Secreted extracellular matrix protein which binds calcium; involved in signal transduction likely via calcium ion binding; may play roles in lymphocyte adhesion to endothelium, extracellular matrix synthesis and cellular adhesion to extracellular matrix, fibrillar collagen formation, "matrix response" in remodeling	Proteinaceous extracellular matrix; Extracellular space; Calcium ion and protein binding; Signal transduction
VSIG4	V-set and immunoglobulin domain-containing 4 protein	Structurally related to B7 family of immune regulatory proteins; negative regulator of T-cell proliferation and interleukin-2 production; inhibitor of alternative complement pathway convertases	Integral to membrane
F8	Coagulation factor VIII, procoagulant component	Participates in the intrinsic pathway of blood coagulation; factor VIII is a cofactor for factor IXa which, in the presence of Ca ²⁺ and phospholipids, converts factor X to the activated form Xa; super-pathways include Formation of fibrin clot (clotting cascade) and complement and coagulation cascades	Oxidoreductase activity; Copper ion binding; Extracellular region