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Transcriptomic profiling of the canine tachycardia-induced heart failure model: global comparison to human and murine heart failure

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Abstract

Alterations of cardiac gene expression are central to ventricular dysfunction in human heart failure (HF). The canine tachycardia pacing-induced HF model is known to reproduce the main hemodynamic, echocardiographic and electrophysiological changes observed in human HF. In this study, we use this HF model to compare gene expression profiles in the left and right ventricles (LV, RV) of normal and end-stage failing canine hearts and compare the transcription profiles to those in human and murine models of HF. In end-stage HF, the LV exhibits down regulation of genes involved in energy production, cardiac contraction, and modulation of excitation–contraction coupling as compared with normal LV. The majority of transcriptomic changes between normal and end-stage canine HF were shared by the RV and LV. Genes down regulated only in the LV included those involved in aerobic energy production pathways, regulation of actin filament length, and enzyme-linked receptor protein signaling pathways. In normal canine hearts, genes encoding specific components of the contractile apparatus exhibit LV–RV asymmetric expression patterns; in failing hearts, cardiac fetal transcription factors *MEF2* and *MITF* and the stress-responsive transcription factor *ATF4* showed interventricular differences in expression. The comparison among the canine tachypacing, mouse transgenic, and human HF reveals that human disease involves down regulation of genes in a broad range of biological processes while experimentally induced HF is associated with down regulation of energy pathways, and that human ischemic HF and canine HF share a similar over representation of transcriptional pathways in the up regulated genes. This study provides insights into the molecular pathways leading to end-stage tachycardia-induced HF, and into global transcriptomic differences between the animal HF models and human HF.

Keywords

Gene expression; Microarray; Cardiomyopathy; Transcriptome; Tachycardia-induced heart failure; RT-PCR; Gene ontology; Remodeling; Pacing

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Appendix A. Supplementary materials. Transcriptomic profiling of the canine tachycardia-induced heart failure model: global comparison to human and murine heart failure

Supplementary data may be found in the online version at doi:10.1016/j.jmcc.2005.08.002.

1. Introduction

The left and right ventricles of the mammalian heart differ in morphologic features, physiologic function, and response to pathophysiological stressors [1–4]. The functional regulation of transcription is critical for cardiac development, chamber specialization, ventricular function, as well as the pathogenesis of heart diseases [1,5–7]. Hence, understanding of differences in the transcriptomes of the left (LV) and right ventricles (RV) in both normal and diseased states is essential to unveiling molecular mechanisms of normal and pathologic ventricular function. Furthermore, the differential responses of the right and left heart to insults that culminate in systolic failure remain unexplored.

The canine tachycardia pacing-induced HF model is a highly relevant large mammalian model that mimics the fundamental mechanical, electrophysiological, biochemical and molecular changes in human HF [8,9]. Although the canine tachycardia HF model has been used to characterize pathophysiological changes in HF and to explore the roles of individual genes/proteins in pathogenesis of HF [9–14], genome-wide expression analyses to identify pathways/networks that underlie the molecular genesis of the HF phenotype in this model have not been performed.

Microarray-based gene expression profiling provides a robust approach for characterizing transcriptomic changes associated with complex pathophysiological phenotypes by simultaneously measuring expression of thousands of genes. Microarray technology has been utilized to identify differentially expressed genes in terminally failing vs. non-failing human hearts as well as mouse transgenic HF models [15–22]. However, little has been done to systematically compare expression profiles between various animal models and human HF of different etiologies. Comparisons of the global transcriptomic profiles of human HF of different etiologies and animal models of HF induced by various interventions will provide important insights regarding the validity of the translation of findings from animal models to human HF.

This study examines transcriptome-scale expression profiling of the LV and RV from normal canines and those rapidly paced into terminal heart failure. First, this study aims to explore molecular mechanisms underlying tachycardia-induced HF. Second, we are interested in understanding interventricular heterogeneity between normal and end-stage HF. Third, we will compare gene expression profiles of the canine tachycardia-induced HF model with mouse transgenic HF model and human HF of different etiologies.

2. Materials and methods

The overall experimental design used four dogs for normal hearts and four dogs paced into end-stage heart failure. The dogs are male mongrels of similar age and size. LV and RV were obtained for gene expression profiling from the eight animals. RNA from LV and RV of each animal was labeled with Cy5 or Cy3, respectively, then hybridized to a microarray. A Dye-swap was conducted for each animal. Four comparisons were performed in data analysis: normal LV vs. normal RV, normal LV vs. failing LV, normal RV vs. failing RV, and failing LV vs. failing RV. This experimental design incorporates four biological replicates and four technical replicates (two dye-swaps).

2.1. Pacing-induced heart failure protocol

Left and right ventricles were obtained from four normal and four failing age-matched male canine hearts. Heart failure was induced by rapid pacing from the RV following the protocols approved by Johns Hopkins University Animal Care and Use Committee [23]. Briefly, animals were intubated and anesthetized; blood oxygen saturation and expired CO₂ were kept within the physiological range throughout the procedure. A VVI pacemaker (Medtronic Inc,

Minneapolis, MN) was implanted via a left lateral thoracotomy and the pacing lead was fixed to the RV epicardium under sterile conditions. Rapid pacing at 240 ppm was initiated 1–2 days after surgery and maintained for approximately 4 weeks. Terminal heart failure was verified by hemodynamic measurements (LV end-diastolic pressure, dP/dtmax and min) at the time of sacrifice. Hearts were harvested by left lateral thoracotomy and immersed in ice-cold saline, tissue sections were quickly frozen in liquid nitrogen. Tissue samples from the hearts of four unoperated normal dogs were similarly obtained. We have previously shown that sham operated and unoperated controls exhibit similar mechanical, neurohumoral and electrophysiological phenotypes, thus to reduce cost and morbidity for the animals unoperated controls were used [9,11,23,24]. The canine pacing tachycardia model is characterized by systolic LV failure, preserved RV systolic function and no significant inflammation or fibrosis [24].

2.2. RNA isolation, microarray hybridization, and data acquisition

Total RNA was prepared from canine ventricular tissues using Trizol reagent (GibcoBRL) and Qiagen RNeasy column (Qiagen). Eight micrograms of total RNA from the LV and RV of each dog was used to synthesize the first strand cDNA in the presence of Cy3 or Cy5 dUTP, respectively. After purification with Qiagen's MinElute PCR Purification Kit, the labeled cDNA from LV and RV was hybridized to human 20 K cDNA microarrays. The human 20 K cDNA microarray was fabricated by the Microarray Core of the Johns Hopkins University Sidney Kimmel Comprehensive Cancer Center. Cross-species hybridization has been shown to be a feasible approach for expression profiling of orthologous genes [25]. For canine, comparative genomic analysis between canine and human has shown that more than 25% of canine survey genomic sequences align uniquely to the human genome, and that 83% of human transcripts align to canine genomic sequences for more than 50% of their lengths [26]. Hence, human cDNA microarrays are a feasible platform for canine gene expression profiling. We used a relatively high stringency hybridization and washing to reduce cross-hybridization (see supplement methods). An average 47% of 20 K probes on the microarrays had signal intensities greater than twice the background signal over the entire experiment. Fluorescent signal intensities were measured using a GenePix 4000 A fluorescent scanner (Axon Instruments), and signal intensity data were extracted using GenePix 4.0 microarray analysis software. For each array, we plotted signal intensities of Cy5 and Cy3 against each other to examine linearity of the correlation between two dyes, an indication of channel saturation. In this study, two dye-swap hybridizations were conducted for each heart with RNA samples from the LV and RV labeled with Cy5 and Cy3, respectively.

2.3. Real-time PCR validation

Primer and probe sets were designed, using the Primer Express Software (Applied Biosystems), for a subset of differentially expressed genes identified in this study. Total RNA samples were prepared from canine ventricular tissues for RT-PCR studies using Qiagen RNeasy with on-column DNase digestion. Fluorescence-based kinetic real-time PCR was conducted with a Perkin-Elmer Applied Biosystems Model 7900 sequence-detection system. 18S ribosomal RNA was used as internal reference, and the signal is divided by the signal of the internal reference to normalize for non-PCR-related fluorescence.

2.4. Microarray data analysis

A ratio-intensity (RI) plot was generated for each array to determine dependency of the log ratios of the two channels on signal intensity by plotting the log ratio against the average of log intensity for Cy3 and Cy5 measurements from each spot (data not shown). We used the significance analysis of microarrays (SAM) software to identify genes differentially expressed between the experimental groups with high statistical significance [27]. The SAM method

provides the false discovery rate (FDR) estimate, using permutations of repeated measurements, to a set of gene-specific *t*-tests (<http://www-stat.stanford.edu/~tibs/SAM>). A two-stage linear mixed model [28] was used to identify differentially expressed genes in the following comparisons: normal LV vs. normal RV; and HF LV vs. HF RV. In the two-stage linear mixed model, the normalization model was used to remove bias due to experiment-wide systematic effects; a gene-specific model was used for gene-by-gene inference of effects such as individual subjects, ventricular chambers (LV, RV), stage (normal, end-stage HF), and chamber–stage interaction (see supplement methods).

Expression Analysis Systematic Explorer (EASE) was used to analyze the biological themes—over representation of genes in a Gene Ontology GO class is present if a larger fraction of genes within that go class is differentially expressed compared with all genes on the array [29]. To compare gene expression profiles of the canine tachycardia-induced HF with those of mouse HF and tissues from HF patients, mouse expression data were obtained from the *TNF- α* over expression-induced HF [20] and the muscle LIM protein gene knockout (*MLP*^{−/−}) murine models [21]; the gene expression data from human idiopathic and ischemic HF were downloaded from the website of Cardiogenomics (www.cardiogenomics.med.harvard.edu), a Program for Genomic Applications (PGA) funded by the National Heart, Lung, and Blood Institute (NHLBI). The differentially expressed genes for EASE analysis were identified using SAM, respectively, from each of datasets in this study.

3. Results

3.1. Evaluation of hemodynamic state

Rapid ventricular pacing consistently produced clear hemodynamic signs of systolic HF as evidenced by an elevated left ventricular end-diastolic pressure (LVEDP > 25 mmHg), a depressed maximum rate of change of left ventricular pressure ($+dP/dt_{\max} < 1700$ mmHg/s), a delayed time constant of relaxation (> 45 ms), and clinical symptoms of HF, such as lethargy, loss of appetite, dyspnea, and/or ascites when compared with control animals (Table 1).

3.2. Differential gene expression patterns between the normal and failing LV

Comparisons between normal and failing LV using SAM identified 1020 differentially expressed genes, including 971 and 49 genes down and up regulated in the failing LV, respectively (Tables S1, S2). EASE software was used to identify statistically significant over representation of transcripts in the GO categories of cellular component and biological processes among the genes differentially expressed between normal and failing LV, which may identify the predominant biological pathways or/and cellular locations associated with the transcriptomic changes. Significance thresholds of EASE score ≤ 0.05 and within-system FDR ≤ 0.01 were employed (Table 2). The genes with a higher expression in failing LV were associated with biological processes involving nucleic acid metabolism, transcription, and regulation of transcription. The down regulated genes in failing LV were largely associated with energy pathways and monovalent inorganic cation transport. This result suggests that the end-stage failing LV is characterized by down regulation of genes related to energy production in mitochondria and cytoplasm and up regulation of genes involved in transcriptional regulation. Furthermore, the genes encoding the cardiac contractile apparatus and mediating excitation–contraction (E–C) coupling were down regulated in the end-stage failing LV (Table 3). These results suggest that the transcriptional dysregulation of energy pathways, contractile apparatus, cell–cell junction, and E–C coupling is associated with deterioration of LV function.

3.3. Interventricular heterogeneity in differential gene expression between normal and end-stage failing hearts

1109 genes were differentially expressed in normal and failing RV, including 52 up regulated and 1057 down regulated genes in HF, based on SAM analysis (Table S3, S4). EASE analysis revealed that the genes up regulated in HF were associated with nucleic acid metabolism, transcription and regulation; the down regulated genes in HF were involved in energy generation pathways, protein modification, hydrogen transport, cell proliferation, and protein metabolism (Table 2). We compared the differentially expressed genes in the normal vs. failing RV and LV to characterize interventricular differences in transcriptomes in normal and end-stage failing hearts. The overwhelming majority (96%) of the genes up regulated in HF were the same in LV and RV. Moreover, ~55% of all of the genes down regulated in HF were shared by both the LV and RV. The differentially expressed genes in HF that were unique to either the LV or RV were further analyzed with EASE to better understand interventricular functional differences. The LV-specific down regulated genes in HF were over represented in the functional categories of oxidative phosphorylation, regulation of actin filament length, enzyme-linked receptor protein signaling pathway, and ATP synthesis coupled electron transport (Table 4, see Table S6 for the list of 410 LV-specific genes). The RV-specific genes that were down regulated in HF were linked to metabolism especially nucleic acid metabolism and the cell components vesicle and nucleus (Table 4, see Table S7 for the list of 422 RV-specific genes). These results suggest that majority of differentially expressed genes in failing compared with normal hearts were shared in both ventricles, with the exception of genes related to energy utilization pathways which were preferentially dysregulated in the LV.

3.4. Interventricular differences in gene expression in normal and end-stage hearts

Gene expression was compared between LV and RV in normal and end-stage failing hearts, respectively. SAM analysis identified a large number of differentially expressed genes but with a higher FDR than the comparisons between normal and failing hearts. A linear mixed model was used to assess specific effects that resulted from biological variability between individual dogs, ventricular chambers, and disease stage. Fifty-two genes were identified to be differentially expressed between the LV and RV of normal canine hearts. Among these, 28 genes were expressed at a higher level in the LV ($P < 0.005$ and fold > 1.15); 24 genes were expressed at a lower level in the LV ($P < 0.005$ and decrease $> 15\%$) (Table S5). With EASE score ≤ 0.05 and within-system FDR ≤ 0.01 , no biological processes or cell components were over represented in the differentially expressed genes. The differentially expressed genes are involved in cell growth and cell cycle regulation, cell adhesion, signal transduction pathways, and metabolism. In addition, it is noteworthy that four and a half LIM domains 2 (*FHL2*) were down regulated in LV.

Seventeen and 14 genes were expressed at a higher and lower levels, respectively, in the LV compared with RV in end-stage heart failure based on the above criteria (Table S5). EASE analysis did not identify over represented GO biological processes and cell components with EASE score ≤ 0.05 and within-system FDR ≤ 0.01 . *MEF2 A* and *MITF*, fetal cardiac transcription factors, exhibited reduced expression in the failing LV compared with the failing RV. Conversely, activating transcription factor 4 (*ATF4*), an ER-stress-responsive transcription factor, exhibited greater expression in the LV than in the RV, implying differential interventricular stress responses in the failing heart.

3.5. Quantitative RT-PCR validation

Quantitative RT-PCR was performed on genes from the four comparisons: normal LV vs. HF LV, normal RV vs. HF RV, normal LV vs. normal RV, and HF LV vs. HF RV (Fig. 1). The direction (up- or down regulation) of the RT-PCR results agreed with that of the microarray results except *MLC2A* in the comparison of failing LV and RV. In general, the results from

the two methods showed consistency in the direction of change in expression, without consistent overestimation by either method of differentially expressed genes.

3.6. Canine HF vs. human and murine HF

We compared canine HF (tachycardia-pacing) with human HF (idiopathic and ischemic) and mouse HF (*TNF- α* overexpression and *MLP*^{-/-}) based on GO functional categorization of differentially expressed genes that were identified from this study, human and mouse gene expression datasets (see Section 2). For each of the datasets, EASE was used to identify the GO categories of cellular component and biological processes that are over represented among differentially expressed genes between normal and HF (Tables 5a and 5b). An EASE score ≤ 0.05 and within-system FDR ≤ 0.01 were employed as cutoff. This analysis intends to reveal transcriptomic relationship between canine, mouse, and human HF based on “biological theme” instead of individual genes.

Table 5a summarizes the GO cellular components that are over represented in the genes up- and down regulated in different types of HF. In HF, genes localized in the nucleus are over represented in the up regulated genes in canine HF and human ischemic HF; genes associated with extracellular matrix are over represented among the up regulated genes in human idiopathic HF. The up regulated genes in mouse *TNF* overexpression-induced HF are related to the vacuole, lysosome, and caspase complex; the up regulated genes in mouse *MLP*^{-/-}-induced HF are linked to the extracellular matrix, vacuole, lysosome, and endoplasmic reticulum. Among the genes down regulated in HF, those expressed in mitochondria are over represented in the experimentally induced canine and mouse HF; human ischemic and idiopathic HF down regulated genes are linked to cellular components involved in protein translation and cytoskeleton.

Table 5b compares the GO biological processes that are over represented in the genes up- and down regulated in canine, mouse and human HF. Genes associated with nucleic acid metabolism, transcription and its regulation are over represented in the genes up regulated in canine and human ischemic HF; human idiopathic HF exhibits up regulation of genes related to development, perception and response to stress, cell adhesion, and ion homeostasis. In mouse *TNF* overexpression-induced HF, a spectrum of *TNF*-mediated biological processes are over represented among the up regulated genes in the failing hearts. Moreover, genes related to cellular ion homeostasis, blood coagulation, and cell growth are over represented in the up regulated genes in mouse *MLP*^{-/-} induced HF. For down regulated genes in HF, the experimentally induced canine and mouse HF show similar over representation of energy pathways. On the other hand, the down regulated genes in human HF involve more diverse biological processes. Human ischemic HF is linked to over representation of genes involved in protein biosynthesis/metabolism, muscle development/contraction, cell motility, cytoplasm organization and biogenesis, and vesicle-mediated transport. Human idiopathic HF displays down regulation in an even broader range of biological processes from nucleic acid metabolism to RNA processing and to protein metabolism.

4. Discussion

Canine tachycardia pacing-induced HF closely mimics the hemodynamic, structural and electrophysiological changes in failing human hearts [8,9]. The hemodynamic hallmark of end-stage HF is a reduction in contractile performance. In this study, we observed a series of transcriptional changes that provide insights into the mechanism of inotropic deterioration in end-stage HF. GO-based EASE analysis demonstrated that genes down regulated in the failing LV were significantly associated with energy pathways in both mitochondria and cytoplasm (Table 2). This suggests that the LV experiences cellular energy stress in end-stage HF. The energy imbalance resulting from impaired energy production may worsen by increased energy

demand due to inefficient sarcomeric ATP utilization, perhaps further undermining ventricle function [30].

The genes expressed in the contractile apparatus and cell–cell junctions were down regulated in the failing LV. Liew and Dzau (2004) proposed “cytoskeletalopathy”, “sarcomyopathy”, and “cytokinopathy” as potential molecular mechanisms of human HF, based on gene expression profiling of human HF [15]. Our results are consistent with “cytoskeletalopathy” and “sarcomyopathy” of the LV in tachypacing-induced HF. The down regulated genes in the failing LV encode structural proteins, including components of contraction and relaxation machinery of the cardiomyocyte such as actin, myosin, troponin T2, troponin I, dynein, capping protein (actin filament) muscle Z-line, cytoskeleton-associated protein 1, profilin 2 and spectrin β (Table 3). The down regulated genes of the cytoskeleton include: the genes encoding components of the costamere such as syntrophin β 2, utrophin, integrins, and cadherins; the gene for sarcolemmal-associated protein; and the genes for tubulins involved in the microtubular network (Table 3). Genes encoding proteins involved in cell–cell junctions were also down regulated in end-stage HF, including desmocollin 2, plakoglobin, desmoplakin, tissue inhibitor of metalloproteinase 3, and integrins (Table 3). The transcriptional down regulation of structural and cell–cell junction proteins contribute to the dysfunctional contraction, relaxation and force transmission in end-stage failing heart.

The down regulation of genes involved in ion homeostasis and signal transduction in end-stage HF is consistent with the well-described alterations in electrophysiology, calcium handling and signaling that characterize the end-stage failing heart (Table 3). A number of genes central to calcium and hypertrophic signaling are down regulated in the failing LV. The ryanodine receptor 2 (*RYR2*) is responsible for calcium release from sarcoplasmic reticulum critical to EC-coupling [31]. Phospholemman has been shown to regulate contractility, $[Ca^{2+}]$ transients and Na^+/Ca^{2+} exchange [32,33]. Calcineurin is a Ca^{2+} /calmodulin-regulated serine/threonine protein phosphatase involved in the WNT pathway for cardiac development and multiple signaling pathways leading to cardiac hypertrophy [34,35]. The genes for calcium/calmodulin-dependent protein kinase, cAMP-dependent protein kinase, and protein kinase C, all down regulated in end-stage HF, are involved in regulation of cardiac contraction and relaxation through regulation of intracellular $[Ca^{2+}]$. These data demonstrate that the transcriptional changes in the end-stage failing LV adversely impact energy production, function of contractile apparatus, the integrity of cell–cell junctions, and ion homeostasis.

There is limited information on the differences in gene expression in normal and diseased LV and RV. To characterize the changes in the LV and RV transcriptomes, we compared the differentially expressed genes identified, respectively, from two comparisons: normal LV vs. failing LV and normal RV vs. failing RV. Based on EASE analysis, genes localized in mitochondria and cytoplasm and genes in energy pathways are similarly over represented in the lists of down regulated genes in both failing LV and RV; on the other hand, genes localized in nucleus and genes related to transcription and its regulation are similarly over represented in the genes up regulated in failing LV and RV (Table 2). The over represented genes down regulated only in the LV are linked to biological processes such as oxidative phosphorylation, ATP synthesis coupled electron transport, carbohydrate metabolism, regulation of actin filament length (Table 4). This analysis suggests that although the LV and RV undergo similar transcriptional changes in HF, the LV experiences more profound energy stress in this model of HF.

One of the goals of this study is to identify interventricular differences in gene expression in normal and end-stage failing hearts. In normal hearts, cardiac α actin (*ACTC*) and myosin light chain 2 (*MRLC2*) showed a lower expression in LV compared with RV. Also, the cardiac transcription factor *FHL2*, which modifies the β -adrenergic stimulation-mediated hypertrophic

response [36], was down regulated in LV. In end-stage failing hearts, genes for transcription factors showed differential expression between LV and RV. *MEF2A*, related to hypertrophic responses to hemodynamic stress [37], was down regulated in failing LV compared with failing RV. This study has also shown that expression of *MEF2A* was down regulated in the end-stage failing LV when compared with normal LV. It suggests that expression of *MEF2A*, which may be an adaptive response in hypertrophy, is diminished in the LV in end-stage HF. On the other hand, *ATF4*, a stress-responsive transcription factor, was up regulated in the LV. It has been recognized that many stress pathways, including ER-stress (unfolded proteins in their endoplasmic reticulum) and oxidative stress, converge to activate *ATF4* [38]. This indicates that, as compared with the RV, the LV exhibits heightened *ATF4* activation in response to a wide range of stresses in end-stage HF. In aggregate, compared with the RV, the end-stage failing LV might be characterized by the switch of stress-responsive machinery from compensating for hypertrophic stress to dealing with stresses such as ER-stress and oxidative stress.

Understanding the differences between the experimentally induced animal HF and human HF is essential to assess the rationale and limitations of translating discoveries from animal models to human disease. Utilizing a transcriptome-scale approach, we examined biological processes and cellular components over represented in differentially expressed genes between normal and failing hearts in canine tachypacing-induced HF, mouse *TNF- α* overexpression-induced HF, mouse *MLP*^{-/-} induced HF, and human ischemic and idiopathic HF (Tables 5a,b). Characteristics differentiating experimentally induced HF and human HF are that energy pathways are over represented in the genes down regulated in experimentally induced HF while human HF involves down regulation of a broader spectrum of biological processes, prominently related to RNA processing and translation. This suggests that chronically developing human HF and possibly its treatment may impact more biological processes than the experimentally induced genetic or sub-acute HF models. Moreover, the biological processes dominant in the genes up regulated in the two mouse HF models are largely related to modification of the target genes, suggesting that multiple molecular mechanisms converge on a HF phenotype. The genes up regulated in canine HF and human ischemic HF share a similar over representation of transcription and its regulation. Human idiopathic HF involves up regulation of multiple biological processes associated with stress perception and response, development, cell adhesion, and ion homeostasis. Convergent biological processes are regulated in the failing mammalian heart; overlapping regulated pathways are more common to human and large animal model heart compared with genetically induced ventricular dysfunction in rodents.

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Fig 1.

Comparison between microarray and RT-PCR results (NLV: normal LV, NRV: normal RV, HLV: failing LV, and HRV: failing RV).

Table 1

Hemodynamic variables measured in normal and end-stage failing canine hearts

	Normal	End-stage HF
End-diastolic pressure (EDP)	4.00 ± 0.28	29.87 ± 2.81
Maximum rate of change of left ventricular pressure (dP/dTmax)	3302.0 ± 240.4	1477.3 ± 415.1

Table 2

Gene ontology-based EASE analysis of differentially expressed genes between normal and end-stage HF in LV and RV

GO system	LV: up regulated in HF		LV: down regulated in HF		RV: up regulated in HF		RV: down regulated in HF	
	Gene category	EASE score	Gene category	EASE score	Gene category	EASE score	Gene category	EASE score
Biological processes	nucleobase/, nucleoside/, nucleotide and nucleic acid metabolism	0.00046	energy pathways	2.46E-06	Nucleobase/, nucleoside/, nucleotide and nucleic acid metabolism	0.00046	metabolism	4.83E-05
	transcription/, DNA-dependent	0.00050	energy derivation by oxidation of organic compounds	3.70E-05	transcription/, DNA-dependent	0.00050	physiological process	0.00049
	transcription	0.00063	main pathways of carbohydrate metabolism	0.00058	transcription	0.00063	energy pathways	0.00060
	regulation of transcription/, DNA-dependent	0.00198	carbohydrate metabolism	0.00480	regulation of transcription/, DNA-dependent	0.00198	protein modification	0.00549
	regulation of transcription	0.00223	alcohol metabolism	0.00831	regulation of transcription	0.00223	hydrogen transport	0.00934
Cellular component			monovalent inorganic cation transport	0.00964			cell proliferation	0.00979
			aerobic respiration	0.00993			energy derivation by oxidation of organic compounds	0.01118
			cellular respiration	0.00993			protein metabolism	0.01206
	nucleus	0.01475	cytoplasm	1.13E-05	Nucleus	0.01475	mitochondrion	0.00066
			mitochondrion	0.00041			cytoplasm	0.00561
			intracellular integral to plasma membrane	0.01174			intracellular	0.01453
			inner membrane	0.01572				
				0.02918				

Down regulated genes related to cardiac contractile and contraction modulation in end-stage failing LV

Table 3

Gene product	Gene symbol	Chromosome	Fold
Contractile apparatus	Myosin, heavy polypeptide 8, skeletal muscle	MYH8	17p13.1
	Myosin, light polypeptide 4, alkali; atrial, embryonic	MYL4	17q21-qter
Cell-cell junction	Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	MYL6	
	Myosin, light polypeptide 3, alkali; ventricle		
	Actin α cardiac muscle	ACTC	
	Actin β		
	Capping protein (actin filament) muscle Z-line, alpha 1	CAPZA1	1p36.13-q23.3
	Capping protein (actin filament) muscle Z-line, beta	CAPZB	1p36.1
	Troponin I, skeletal, fast	TNNI2	11p15.5
	Troponin T2, cardiac	TNNI2	1q32
	Dynein, cytoplasmic, intermediate polypeptide 2	DNCL2	7q21.3-q22.1
	Cytoskeleton-associated protein 1	CKAP1	19q13.11-q13.12
Cardiac contraction modulation	Profilin 2	PFN2	3q25.1-q25.2
	Spectrin, beta, non-erythrocytic 1	SPTBN1	2p21
	Sarcolemmal-associated protein	SLAP	3p21.2-p14.3
	Syntrophin, beta 2 (dystrophin-associated protein A1, 59kD, basic component 2)	SNTB2	16q22-q23
	Utrophin (homologous to dystrophin)	UTRN	6q24
	Tubulin, beta polypeptide	TUBB	6p21.3
	Tubulin, gamma 2	TUBG2	17q21
	Integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)	ITGA3	
	Integrin, alpha 7	ITGA7	12q13
	Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)	ITGAV	2q31-q32
Cardiac contraction modulation	Junction plakoglobin	JUP	17q21
	Desmocollin 2	DSC2	18q12.1
	Desmoplakin (DPI, DPII)	DSP	6p24
	Tissue inhibitor of metalloproteinase 3	TIMP3	22q12.3
	laminin, alpha 3	LAMA3	18q11.2
	Ryanodine receptor 2 (cardiac) (RYR2), mRNA	RYR2	
	calcineurin A gamma	PPP3CC	
	calcineurin B, type I	PPP3R1	
	Calcium/calmodulin-dependent protein kinase		2p15
	Phospholemman		
Cardiac contraction modulation	Calcineurin binding protein 1	KIAA0330	22q11.23
	Calnexin	CANX	5q35
	mitogen-activated protein kinase 9	MAPK9	5q35
	mitogen-activated protein kinase 2	MAP2K2	7q32
	mitogen-activated protein kinase kinase 4	MAP2K4	17p11.2
	mitogen-activated protein kinase kinase 11	MAP3K11	11q13.1-q13.3
	mitogen-activated protein kinase 8		
	protein kinase, cAMP-dependent, catalytic, beta	PRKACB	1p36.1
	protein kinase, cAMP-dependent, regulatory, type I, alpha	PRKAR1A	17q23-q24
	protein kinase C, mu	PRKCM	14q11
Cardiac contraction modulation	protein kinase C, zeta	PRKCZ	1p36.33-p36.2
	angiotensin II, type I receptor-associated protein	ATRAP	
	endothelin converting enzyme 1	ECE1	1p36.1
	Myoglobin	MB	22q13.1
			0.34528
			0.17737
			0.43206
			0.43957
			0.22944
			0.11516
			0.16790
			0.42011
			0.52853
			0.29962
			0.25785
			0.18158
			0.18870
			0.48402
			0.35422
			0.43727
			0.14915
			0.27842
			0.30177
			0.38721
			0.26000
			0.31595
			0.42314
			0.35512
			0.36978
			0.44588
			0.46139
			0.15571
			0.6q24
			6p21.3
			17q21
			0.44588
			0.36978
			0.35512
			0.42314
			0.31595
			0.26000
			0.38721
			0.30177
			0.27842
			0.14915
			0.35422
			0.43727
			0.48402
			0.18870
			0.18158
			0.25785
			0.29962
			0.52853
			0.42011
			0.16790
			0.11516
			0.22944
			0.43957
			0.43206
			0.17737
			0.09920
			0.04816
			0.34528

Table 4
GO-based EASE analysis of LV- and RV-specific down regulated genes in end-stage HF

GO system	Down regulated only in failing LV	EASE score	Down regulated only in failing RV	EASE score
Biological process	energy derivation by oxidation of organic compounds	0.00116	Metabolism nucleobase/, nucleoside/, nucleotide and nucleic acid metabolism	0.00293
	energy pathways	0.00510		0.01646
	oxidative phosphorylation	0.00515		
	main pathways of carbohydrate metabolism	0.00727		
	regulation of actin filament length	0.01104		
Cellular component	enzyme-linked receptor protein signaling pathway	0.01141	Clathrin coat Clathrin vesicle coat Vesicle coat membrane coat Nucleus	
	protein-disulfide reduction	0.01918		
	ATP synthesis coupled electron transport	0.01918		
	cytoplasm	0.01279		0.01698
				0.01698
				0.02268

GO biological processes categories of differentially expressed genes between normal and HF in the mouse transgenic models, canine tachypacing model and human HF

Table 5b

Mouse TNF overexpression	Mouse MLP-/-	Up regulated in HF Human Idiopathic	Human ischemic	Canine tachypacing	Mouse TNF overexpression	Mouse MLP-/-	Down regulated in HF Human idiopathic	Human ischemic	Canine tachypacing
Defense response	Cell ion homeostasis	Development	Nucleobase/nucleoside/nucleotide and nucleic acid metabolism Transcription/, DNA dependent	Nucleobase/nucleoside/nucleotide and nucleic acid metabolism Transcription/, DNA dependent	Main pathways of carbohydtrate metabolism	Fatty acid metabolism	Protein metabolism	Protein biosynthesis	Energy pathways
Immune response	Ion homeostasis	Perception of abiotic stimulus	Transcription/, DNA dependent		Oxidative phosphorylation	Lipid metabolism	Intracellular protein transport	Protein metabolism	Energy derivation by oxidation of organic compounds
Inflammatory response	Blood coagulation	Sensory perception	Transcription	Transcription	TCA cycle	Proton transport	Ubiquitin dependent protein catabolism	Muscle contraction	Main pathways of carbohydtrate metabolism Alcohol metabolism
Antigen processing	Transition metal ion homeostasis	Perception of external stimulus	Regulation of transcription/, DNA dependent	Regulation of transcription/, DNA dependent	Energy derivation by oxidation of organic compounds	Carbohydrate metabolism	Protein modification	Muscle development	
Antigen presentation	Cation homeostasis	Cell adhesion	Regulation of transcription	Regulation of transcription	Energy pathways	Electron transport	Nucleobase/nucleoside/nucleotide and nucleic acid metabolism RNA processing	Cell motility	Monovalent inorganic cation transport
Cell proliferation	Cell homeostasis	Metal ion homeostasis	Regulation of transcription from Pol II promoter RNA processing	Regulation of transcription	ATP synthesis coupled electron transport	ATP biosynthesis metabolism		Regulation of translational initiation	Aerobic respiration
Chemotaxis	Cell growth	Response to radiation	RNA metabolism		Protein disulfide reduction	Energy pathways	Cell growth and maintenance	Translational initiation	Cellular respiration
Cell death					Mitochondrial electron transport	Glucose catabolism	Nucleo-cytoplasmic transport	Translational translation	
Lymph gland development					Cation transport	Amino acid catabolism	Phosphorus metabolism	Cytoplasm organization and biogenesis	
Cell cycle					Hydrogen transport	Ribo-nucleotide biosynthesis	Vesicle-mediated transport	Vesicle mediated transport	
Endocytosis						Fatty acid oxidation	Small GTPase mediated signal transduction		
Vesicle-mediated transport						Coenzyme biosynthesis	Translation		
Regulation of cell cycle							Protein targeting		
Apoptosis							Negative regulation of transcription		
Cytokinesis							Cell proliferation		
Phagocytosis							Protein folding		
Cell growth							RNA splicing		
Actin filament-based process							Cell cycle		
							Phosphorylation		
							ER to Golgi transport		