Smith College

Smith ScholarWorks

Biological Sciences: Faculty Publications

Biological Sciences

4-22-2008

Human Cardiac-Specific cDNA Array for Idiopathic Dilated Cardiomyopathy: Sex-Related Differences

Georges E. Haddad
Howard University

Lori J. Saunders
Gwathmey, Inc.

Seth D. Crosby
Washington University School of Medicine in St. Louis

Maria Carles
Gwathmey, Inc.

Federica Del Monte
Beth Israel Deaconess Medical Center

See next page for additional authors

Follow this and additional works at: https://scholarworks.smith.edu/bio_facpubs

Part of the Biology Commons

Recommended Citation

Haddad, Georges E.; Saunders, Lori J.; Crosby, Seth D.; Carles, Maria; Del Monte, Federica; King, Kindra; Bristow, Michael R.; Spinale, Francis G.; Macgillivray, Thomas E.; Semigran, Marc J.; Dec, G. William; Williams, Steven A.; Hajjar, Roger J.; and Gwathmey, Judith K., "Human Cardiac-Specific cDNA Array for Idiopathic Dilated Cardiomyopathy: Sex-Related Differences" (2008). Biological Sciences: Faculty Publications, Smith College, Northampton, MA.
https://scholarworks.smith.edu/bio_facpubs/166

This Article has been accepted for inclusion in Biological Sciences: Faculty Publications by an authorized administrator of Smith ScholarWorks. For more information, please contact scholarworks@smith.edu

Authors

Georges E. Haddad, Lori J. Saunders, Seth D. Crosby, Maria Carles, Federica Del Monte, Kindra King, Michael R. Bristow, Francis G. Spinale, Thomas E. Macgillivray, Marc J. Semigran, G. William Dec, Steven A. Williams, Roger J. Hajjar, and Judith K. Gwathmey

This article is available at Smith ScholarWorks: https://scholarworks.smith.edu/bio_facpubs/166

Physiol Genomics 33: 267–277, 2008.
First published February 26, 2008; doi:10.1152/physiolgenomics.00265.2007.

Human cardiac-specific cDNA array for idiopathic dilated cardiomyopathy: sex-related differences

Georges E. Haddad,[1] Lori J. Saunders,[2] Seth D. Crosby,[3] Maria Carles,[2] Federica del Monte,[4] Kindra King,[2] Michael R. Bristow,[5] Francis G. Spinale,[6] Thomas E. Macgillivray,[7] Marc J. Semigran,[8] G. William Dec,[8] Steven A. Williams,[10] Roger J. Hajjar,[9] and Judith K. Gwathmey[2,11]

[1]*Department of Physiology and Biophysics, College of Medicine, Howard University, Washington, District of Columbia;* [2]*Gwathmey, Incorporated, Cambridge, Massachusetts;* [3]*Microarray Core Facility, Washington University Medical School, St. Louis, Missouri;* [4]*Cardiovascular Research Center, Beth Israel Deaconess Medical Center, Boston, Massachusetts;* [5]*Division of Cardiology, School of Medicine, University of Colorado Health Sciences Center, Denver, Colorado;* [6]*Cardiothoracic Surgery, Medical University of South Carolina, Charleston, South Carolina;* [7]*Cardiac Surgery, Edwards Research, and* [8]*Cardiology Division, Gray/Bigelow, Massachusetts General Hospital, Boston and* [10]*Department of Biological Sciences, Smith College, Northampton, Massachusetts;* [9]*Cardiovascular Research Center, Mount Sinai School of Medicine, New York, New York; and* [11]*Boston University School of Medicine, Cambridge, Massachusetts*

Submitted 13 November 2007; accepted in final form 12 February 2008

Haddad GE, Saunders LJ, Crosby SD, Carles M, del Monte F, King K, Bristow MR, Spinale FG, Macgillivray TE, Semigran MJ, Dec GW, Williams SA, Hajjar RJ, Gwathmey JK. Human cardiac-specific cDNA array for idiopathic dilated cardiomyopathy: sex-related differences. *Physiol Genomics* 33: 267–277, 2008. First published February 26, 2008; doi:10.1152/physiolgenomics.00265.2007.—Idiopathic dilated cardiomyopathy (IDCM) constitutes a large portion of patients with heart failure of unknown etiology. Up to 50% of all transplant recipients carry this clinical diagnosis. Female-specific gene expression in IDCM has not been explored. We report sex-related differences in the gene expression profile of ventricular myocardium from patients undergoing cardiac transplantation. We produced and sequenced subtractive cDNA libraries, using human left ventricular myocardium obtained from male transplant recipients with IDCM and nonfailing human heart donors. With the resulting sequence data, we generated a custom human heart failure microarray for IDCM containing 1,145 cardiac-specific oligonucleotide probes. This array was used to characterize RNA samples from female IDCM transplant recipients. We identified a female gene expression pattern that consists of 37 upregulated genes and 18 downregulated genes associated with IDCM. Upon functional analysis of the gene expression pattern, deregulated genes unique to female IDCM were those that are involved in energy metabolism and regulation of transcription and translation. For male patients we found deregulation of genes related to muscular contraction. These data suggest that *1*) the gene expression pattern we have detected for IDCM may be specific for this disease and *2*) there is a sex-specific profile to IDCM. Our observations further suggest for the first time ever novel targets for treatment of IDCM in women and men.

gene expression; heart failure; IDCM markers

APPROXIMATELY FIFTY PERCENT of all heart transplant recipients have idiopathic dilated cardiomyopathy (IDCM). About 4,900,000 Americans (2,400,000 males and 2,500,000 females) have IDCM, the incidence of which approaches 10 per 1,000 individuals after age 65 (3, 4). During the course of the disease, myocardial contractile function steadily decreases. Recent investigations have suggested that there may be myocardial properties and/or hormonal environments unique to women or men that contribute to atypical clinical presentation and clinical outcomes. Despite its being well accepted that women suffering from IDCM often have clinical presentations differing from those of men with a similar cardiac condition, little is known about the etiology of these sex-related differences.

In a study by Carroll et al. (15) examining left ventricle hypertrophy caused by aortic stenosis, women had smaller, thicker-walled ventricles despite outflow obstruction similar to that in men. After diagnosis of nonischemic heart failure, women fare somewhat better than men, but <15% survive beyond 8–12 yr after diagnosis (3, 4). The average survival after diagnosis of end-stage heart failure is 5 yr for the male population.

Most studies on sex-related differences have used animal models. Studies using transgenic murine and/or rat models of heart failure have shown that females have less cardiac remodeling, ventricular dysfunction, and pathological change and an increased survival advantage over males (20, 21, 23, 28, 37, 44, 50, 61, 66). Two such studies have suggested that female sex hormones may play a protective role in heart failure. A female phenotype can be mimicked by the use of estradiol in males (11, 20, 66). Conversely, a murine study using testosterone infusion in ovariectomized transgenic females showed increased cardiac mass and fibrosis (44). An additional study using male mice with cardiac overexpression of β_2-adrenergic receptors showed a reduction in heart failure phenotype from orchiectomy (23). These results suggest an additional contribution by testicular male hormones to the progression of the cardiomyopathic phenotype in specific transgenic models. Despite nonhuman research to date, sex-related differences responsible for the better prognosis of human females with heart failure have not yet been clearly established.

The structural and functional changes that occur in IDCM hearts in both men and women are most likely due to changes in gene and protein expression. In a recent microarray study by Barth et al. (6), a common gene expression signature for dilated cardiomyopathy (DCM) in humans consisting of 27 genes was

Article published online before print. See web site for date of publication (http://physiolgenomics.physiology.org).

Address for reprint requests and other correspondence: J. K. Gwathmey, Boston Univ. School of Medicine, 763 Concord Ave., Bldg. E, Cambridge, MA 02138 (e-mail: gwathmey@earthlink.net).

1094-8341/08 $8.00 Copyright © 2008 the American Physiological Society

Downloaded from journals.physiology.org/journal/physiolgenomics (073.004.114.249) on July 15, 2021.

identified. In this study, sex-related differences that may occur in a DCM-specific gene expression pattern were not addressed. Despite these studies on human tissues and animal models thought to mimic this disease, a clear sex-specific profile for IDCM has yet to be determined. Human cardiac tissue studies have been particularly fraught with discrepancies between research groups, with noted differences from animal models (5, 7, 8, 10, 22, 24, 26, 34, 35, 39, 47, 49, 56, 58, 62, 70, 71). Factors contributing to these discrepancies may include the lack of quality samples from which good-quality RNA can be obtained as well as the lack of access to medical histories. In addition, absent from many studies are normal, sex- and age-matched controls. We have, in the present study, rigorously addressed these issues.

Microarray technology is capable of screening vast numbers of genes, or entire genomes, for differential expression. Several investigators have screened samples on nonspecific genome arrays in order to identify deregulated genes (58). We examined the gene expression profile of diseased myocardium from female and male patients with IDCM and control samples from age- and race-matched donors by means of subtractive hybridization and gene microarray technology. To focus only on genes that are potentially involved in IDCM, we have developed a heart-specific array by performing subtractive hybridization in order to preselect differentially expressed clones. By using this approach, we were able to produce a focused microarray containing both up- and downregulated genes, including rare genes expressed at low levels in the nonfailing and failing human heart. The array was used to test the hypothesis that *1*) IDCM has a specific gene profile and *2*) there are sex-specific differences in the expression profile of a critical set of genes that might be important markers of heart disease in men and women. We have identified putative sex-specific differences in the gene expression pattern that characterize the IDCM state. The gene expression differences in the female samples may be indicative of sex-linked disparities in the pathophysiology and potentially even the pathogenesis of heart failure. Identified markers might then serve to specify proteins that might be used as drug target candidates in female and male heart failure patients.

MATERIALS AND METHODS

Human cardiac samples. Patient consent was obtained from all transplant recipients. Family consent was provided for brain-dead organ donors (normal control subjects). The hearts from brain-dead organ donors with normal cardiac function were not used for transplantation because of cardiac arrest with resuscitation, blood transfusion, or lack of a suitable recipient. The clinical characteristics of the IDCM patients are summarized in Supplemental Table S1.[1] The average age of the five male and six female IDCM patients was 57 ± 6.5 and 48 ± 14.8 yr, respectively ($P > 0.24$), and of the average age-matched nonfailing male and female donors was 58 ± 5.5 and 57 ± 4.5 yr ($P = 0.66$).

At the time of tissue procurement, hearts were excised and placed in Wisconsin cardioplegic solution, placed on ice, and transported to the laboratory. The hearts were examined and sectioned by a cardiologist. Sections were snap frozen. Transplant recipient or donor information was then entered into a secure database.

[1] The online version of this article contains supplemental material.

RNA isolation. Left ventricle tissue was pulverized in liquid nitrogen, placed in TRIzol reagent, and immediately homogenized with a rotor-stator homogenizer. Total RNA was isolated according to the manufacturer's instructions (Invitrogen, Carlsbad, CA) with the following exceptions. An additional extraction with phenol (pH 4.3)-chloroform was performed as well as an additional isopropanol precipitation to further purify the RNA.

For each sample, messenger RNA (mRNA) was purified from 700 μg of total RNA sample with the Poly(A) Pure mRNA Isolation Kit (Ambion, Austin, TX) according to the manufacturer's instructions. In addition, mRNA was ethanol precipitated and washed once with 70% ethanol for purification and concentration.

Microarray production. All contigs representing genes derived from both forward and reverse human subtracted cDNA libraries, identified through NCBI database queries, were chosen for production

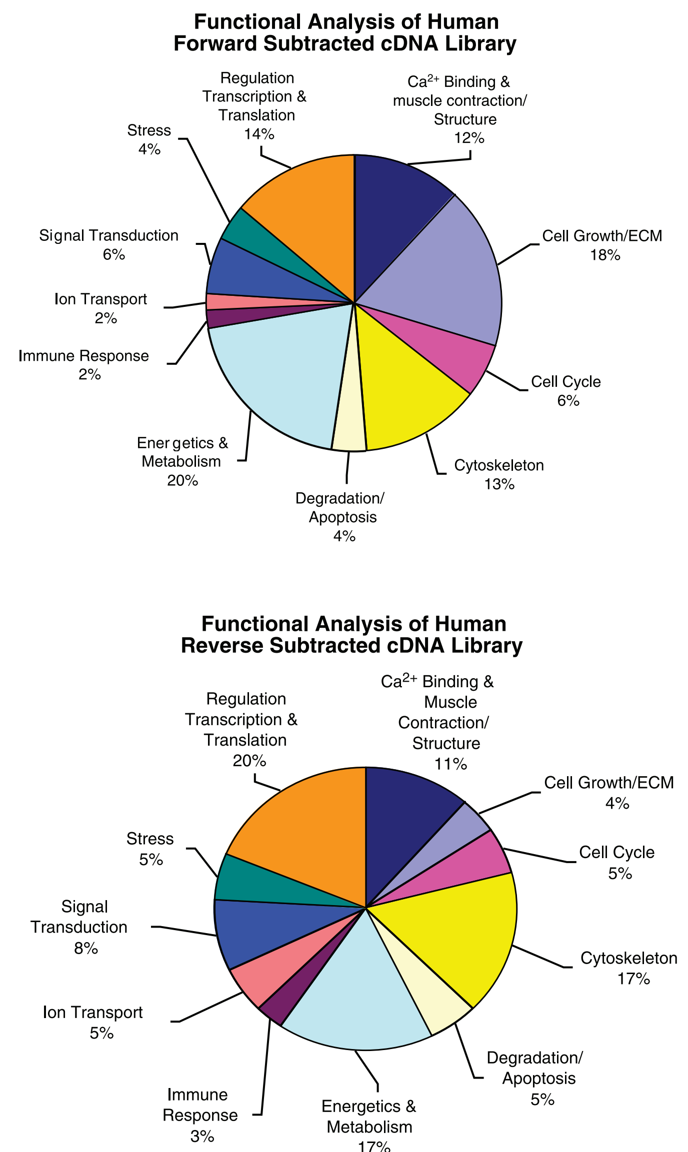

Fig. 1. Functional analysis of genes identified in the forward and reverse subtracted cDNA libraries. Libraries were constructed from pooled male idiopathic dilated cardiomyopathy (IDCM) and pooled male donor left ventricle tissue ($n = 4$ and $n = 10$, respectively). The forward subtracted IDCM cDNA library is enriched for genes that are increased in expression levels or turned on during IDCM. Conversely, the reverse subtracted cDNA library is enriched for genes that are decreased or turned off during IDCM. ECM, extracellular matrix.

Downloaded from journals.physiology.org/journal/physiolgenomics (073.004.114.249) on July 15, 2021.

of a heart failure oligo microarray. One hundred fifteen sequences not identified through subtractive hybridization, but thought to play a role in heart failure according to recent published microarray data (5, 26, 35, 58), were included in the microarray as additional oligonucleotide probes. To construct a gene-specific microarray, we designed 70-mer oligonucleotide array elements. Oligonucleotides were designed from the full-length sequences, if available, with arrayoligoselector (12) and were synthesized by using standard methods by illumina (San

Table 1. *Differentially regulated genes in female IDCM patient samples*

Common Name	Description	Accession No.	Function	Mean Fold Change
		Upregulated genes		
ACO2	**Aconitase 2**	**BC014092**	**Energetics & metabolism**	**6.9**
TTN	**Titin**	**NM_003319**	**Ca^{2+} binding & muscle contraction/structure**	**5.5**
GPI	**Glucose phosphate isomerase**	**NM_000175**	**Energetics & metabolism (carbohydrate)**	**5.1**
HSPA1A	**Heat shock 70-kDa protein 1A**	**NM_005345**	**Stress**	**5.0**
FSTL3	**Follistatin-like 3**	**NM_005860**	**Cell growth/ECM**	**4.0**
IgG1L	Immunoglobulin λ heavy chain	Y14737	Immune response	3.8
HSPCB	**Unknown**	**CN355913**	**Unknown**	**3.5**
NPPA	**Natriuretic peptide precursor A**	**BC005893**	**Regulation of blood pressure**	**3.4**
LGMN	**Legumain**	**NM_005606**	**Degradation/apoptosis**	**2.9**
UBE1	Ubiquitin-activating enzyme E1	BC013041	Degradation/apoptosis	2.9
CD81	CD81 antigen	BC002978	Cell growth/ECM	2.8
Gup1	GRINL1A complex protein 2 precursor	AY208913	Unknown	2.6
NDUFV1	NADH dehydrogenase (ubiquinone) flavoprotein 1	BC015645	Energetics & metabolism	2.4
CTGF	**Connective tissue growth factor**	**NM_001901**	**Cell growth/ECM**	**2.2**
ACTN1	α-Actinin	X15804	Cytoskeleton	2.2
DPT	**Dermatopontin**	**NM_001937**	**Cell Growth/ECM**	**2.1**
RTN4	Reticulon 4	BC010737	Degradation/apoptosis (proapoptosis)	2.1
IGFBP2	Insulin-like growth factor binding protein 2	BC009902	Cell growth/ECM	2.1
LTBP2	Latent transforming growth factor-β binding protein	Z37976	Cell growth/ECM	2.1
COL16A1	**Collagen type XVI α1**	**S57132**	**Cell growth/ECM**	**2.0**
GNB2	Guanine nucleotide binding protein β polypeptide 2	BC010073	Signal transduction	2.0
ACAT1	Acetyl-coenzyme A acetyltransferase 1	NM_000019	Energetics & metabolism (lipid)	2.0
HLA-DRB1	**Major histocompatibility complex class II DR β3**	**BC008403**	**Immune response**	**1.9**
SDHA	Succinate dehydrogenase complex, subunit A, flavoprotein	BC001380	Energetics & metabolism	1.9
HSPA5	Unknown	AL541667	Unknown	1.8
ACTN2	Actinin α2	NM_001103	Cytoskeleton	1.8
MDH1	Malate dehydrogenase 1, NAD (soluble)	NM_005917	Energetics & metabolism	1.8
VDAC3	Voltage-dependent anion channel 3	BC056870	Ion transport	1.8
LOC161247	Similar to CG10671-like	NM_203402	Unknown	1.8
CKM	Creatine kinase muscle	AY335559	Energetics & metabolism	1.7
CSRP1	Cysteine and glycine-rich protein 1	NM_004078	Unknown	1.7
EGFL7	EGF-like-domain	NM_016215	Ca^{2+} binding & muscle contraction/structure	1.7
PCBP2	Poly(rC) binding protein 2	NM_005016	Energetics & metabolism	1.7
PRDX2	Peroxiredoxin 2	BC039428	Stress	1.7
ACTC	Actin, α, cardiac muscle	BC009978	Cytoskeleton	1.6
ACADM	Unknown	CN277078	Unknown	1.5
MYH7	**Myosin heavy chain**	**CF553070**	**Ca^{2+} binding & muscle contraction/structure**	**2.5**
		Downregulated genes		
PLA2G2A	**Phospholipase A_2 group IIA**	**BC005919**	**Energetics & metabolism (lipid)**	**−9.0 (0.11)**
MUSTN1	**Musculoskeletal embryonic nuclear protein 1**	**NM_205853**	**Unknown**	**−3.3 (0.31)**
ADH1B	**Alcohol dehydrogenase IB**	**NM_000668**	**Energetics & metabolism (alcohol)**	**−2.9 (0.35)**
G0S2	**Lymphocyte G_0/G_1 switch gene**	**BC009694**	**Immune response**	**−2.5 (0.40)**
CD36	CD36 antigen	NM_000072	Signal transduction	−1.9 (0.53)
SELENBP1	Selenium binding protein 1	NM_003944	Unknown	−1.9 (0.52)
JUNB	jun B protooncogene	BC009466	Regulation transcription/translation	−1.5 (0.68)
APOD	**Apolipoprotein D**	**NM_001647**	**Energetics & metabolism (lipid)**	**−1.8 (0.56)**
PYGM	**Highly similar to glycogen phosphorylase muscle form**	**AK056607**	**Energetics & metabolism (carbohydrate)**	**−1.8 (0.56)**
FOS	Unknown	BX458870	Unknown	−1.1 (0.90)
CD59	CD59 antigen	NM_203329	Immune response	**−1.7 (0.59)**
MTHFD2	NAD-dependent methylene tetrahydrofolate dehydrogenase cyclohydrolase	X16396	Energetics & metabolism (1-carbon compound)	−1.7 (0.58)
MYL4	Myosin light polypeptide 4, alkali, atrial, embryonic	NM_002476	Ca^{2+} binding & muscle contraction/structure	**−1.7 (0.57)**
GPD1L	Glycerol-3-phosphate dehydrogenase 1	BC028726	Energetics & metabolism (carbohydrate)	−1.7 (0.57)
S100A4	S100 calcium binding protein A4	NM_002961	Ca^{2+} binding & muscle contraction/structure	−1.3 (0.80)
SH3BGRL3	SH3 domain binding glutamic acid-rich protein-like 3	AF247790	Unknown	−1.1 (0.90)
FBLP-1	Unknown	AK130732	Unknown	−1.6 (0.61)
LDB3	LIM domain binding 3	BC010929	Ca^{2+} binding & muscle contraction/structure	−1.5 (0.67)

Genes up- or downregulated (>1.8-fold) in at least 3 of 6 female idiopathic dilated cardiomyopathy (IDCM) patient samples are shown. Fold change represents mean fold change in the 6 female IDCM patients. Genes in bold were also found to be up- or downregulated (>1.8-fold) in at least 3 of 5 male IDCM patient samples. ECM, extracellular matrix.

Downloaded from journals.physiology.org/journal/physiolgenomics (073.004.114.249) on July 15, 2021.

Diego). The oligonucleotides were dissolved at a concentration of 50 mM in 3× SSC with 0.75 M betaine and were printed in duplicate on MWG Epoxy slides (MWG Biotech, Ebersburg, Germany) by a locally constructed linear servo arrayer (after the DeRisi model; Ref. 41). A total of 1,145 genes were represented on the heart-specific microarray along with 6 *Escherichia coli* "ArrayControl" oligonucleotides (Ambion) representing sequences that do not hybridize to mammalian sequences (total 1,151). Oligonucleotides were spotted in triplicate onto epoxy-coated slides obtained from MWG (Germany) and stored at −20°C.

cDNA synthesis and microarray hybridization. cDNA was synthesized from 2 μg of total RNA isolated from left ventricle tissue of each IDCM transplant recipient (n = 11) or pooled male or female nonfailing left ventricle control sample (n = 10 per sex). cDNA synthesis and hybridization were performed with the 3DNA Array 900 Detection System (Genisphere, Hatfield, PA), following the manufacturer's instructions with modifications. A total of two technical replicates for each patient were performed (dye swap) to correct for any bias introduced by the Cy3 and Cy5 fluorescent dendrimers. Slides were scanned immediately after hybridization on a ScanArray Express HT Scanner (PerkinElmer) to detect Cy3 and Cy5 fluorescence. Laser power was kept constant, and photomultiplier tube (PMT) values were set for optimal intensity with minimal background (high-PMT scan). An additional scan was done for each slide with the PMT such that <1% of the elements are saturated (low-PMT scan) to characterize spots that were saturated in the high-PMT scan. Gridding and analysis of images were performed with scanarray software express version 3.0 (PerkinElmer).

Microarray data analysis (normalization). Values corresponding to the fluorescence intensity for each oligonucleotide spot were obtained and imported into GeneSpring 7.1 software (Agilent, Redwood City, CA). Local background fluorescence intensity was subtracted from individual spot fluorescence intensities. The mean signal and control intensities of the on-slide duplicate spots were calculated. A Lowess curve was fit to the log-intensity vs. log-ratio plot. Twenty percent of the data was used to calculate the Lowess fit at each point. This curve was used to adjust the control value for each measurement. If the control channel was lower than 10 relative fluorescence units (RFU), then 10 was used instead. Mean signal to Lowess-adjusted controlled ratios were calculated. The cross-chip averages were derived from the antilog of the mean of the natural log ratios across the two microarrays (technical replicates-dye swaps).

Subtractive hybridization. With the technique of subtractive hybridization, both a forward (IDCM minus nonfailing) and a reverse (nonfailing minus IDCM) subtracted cDNA library were constructed, following the manufacturer's instructions (Clontech, Mountain View, CA). Each library was constructed with pooled mRNA from left ventricle tissue of 6 male IDCM transplant recipients and pooled mRNA from 10 nonfailing age-matched donors. The forward subtracted IDCM cDNA library is enriched for genes that are increased in expression levels or turned on during IDCM. Conversely, the reverse subtracted cDNA library is enriched for genes that are decreased or turned off during IDCM. One thousand clones were randomly chosen from each library, PCR amplified, and sequenced on a single-pass basis to produce an expressed sequence tag (EST) for each clone. An alignment (SeqMan, DNASTAR, Madison, WI) was performed with all EST sequences from each subtracted cDNA library, and a contig (a collection of ESTs representing the same gene) was generated for each gene identified. Sequences were identified through NCBI database queries.

Microarray data filtering. Data were filtered in the following manner. *1*) Oligonucleotide elements that received a "present" call (intensity >200 RFU or local signal/background >2) by the ScanArray software in at least half the patients in either Cy3 or Cy5 were identified (1,037 genes), and all others were excluded from the analysis. *2*) The genes that demonstrated a CV <30% between the dye swaps for 70% of the patients were selected. *3*) Genes that showed >1.8-fold up- or downregulation in three of five male and three of six female transplant recipients compared with the pooled male or female nonfailing control samples, respectively, were selected.

Real-time quantitative RT-PCR. To confirm the expression patterns detected by our microarray, we randomly selected 39 genes from the pool of deregulated genes identified in male and female IDCM samples for further analysis by means of quantitative RT-PCR. The reactions characterized RNA derived from pooled IDCM male (n = 5), pooled female (n = 6), or nonfailing pooled RNA male or female sample (n = 20). Two-step RT-PCR was performed with 10 ng of total RNA per reaction. Triplicate aliquots of each RNA sample were used in the same reactions. All samples were normalized to 18S rRNA as an internal control. For all experimental samples, the relative fold

Table 2. *Differentially regulated genes in both female and male IDCM patient samples*

Common Name	Description	Accession No.	Function
	Upregulated genes		
ACO2	Aconitase 2	BC014092	Energetics & metabolism
TTN	Titin	NM_003319	Ca^{2+} binding & muscle contraction/structure
GPI	Glucose phosphate isomerase	NM_000175	Energetics & metabolism (carbohydrate)
HSPA1A	Heat shock 70-kDa protein 1A	NM_005345	Stress
FSTL3	Follistatin-like 3	NM_005860	Cell growth/ECM
HSPCB	Unknown	CN355913	Unknown
NPPA	Natriuretic peptide precursor A	BC005893	Regulation of blood pressure
LGMN	Legumain	NM_005606	Degradation/apoptosis
CTGF	Connective tissue growth factor	NM_001901	Cell growth/ECM
DPT	Dermatopontin	NM_001937	Cell growth/ECM
COL16A1	Collagen type XVI α1	S57132	Cell growth/ECM
HLA-DRB1	Major histocompatibility complex class II DR β3	BC008403	Immune response
MYH7	Myosin heavy chain	CF553070	Ca^{2+} binding & muscle contraction/structure
	Downregulated genes		
PLA2G2A	Phospholipase A_2 group IIA	BC005919	Energetics & metabolism (lipid)
MUSTN1	Musculoskeletal embryonic nuclear protein 1	NM_205853	Unknown
ADH1B	Alcohol dehydrogenase IB	NM_000668	Energetics & metabolism (alcohol)
G0S2	Lymphocyte G_0/G_1 switch gene	BC009694	Immune response
APOD	Apolipoprotein D	NM_001647	Energetics & metabolism (lipid)
PYGM	Highly similar to glycogen phosphorylase muscle form	AK056607	Energetics & metabolism (carbohydrate)

Genes up- or downregulated (>1.8-fold) in both female (3 of 6) and male (3 of 5) IDCM patient samples are shown.

Downloaded from journals.physiology.org/journal/physiolgenomics (073.004.114.249) on July 15, 2021.

difference of each gene was determined compared with the pooled nonfailing male or female reference sample by means of the $\Delta\Delta C_t$ (threshold cycle) method (Applied Biosystems).

Western blot analysis. Sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) protein levels were assessed by Western blot technique. Two hundred micrograms of total protein was used for each sample isolated from the left ventricular tissue of a human male donor, a human female donor, a human male donor with idiopathic cardiomyopathy, or a human female donor with idiopathic cardiomyopathy. Tissue lysates were matched for protein concentration, separated by SDS-PAGE, and transferred onto nitrocellulose membranes. After blocking in 5% non-fat milk in TBST (10 mM Tris, 150 mM NaCl, 0.1% Tween 20), the membranes were incubated with specific antibodies to SERCA2a (Cell Signaling Technology) overnight at 4°C. Afterward, membranes were washed three times in TBST, incubated with appropriate secondary antibody conjugated to horseradish peroxidase (Cell Signaling Technology) for 2 h, and then washed three times with TBST. Bands were visualized by Chemiluminescence (Renaissance, NEN Life Science Products). Relative specific protein levels between IDCM and nonfailing donor samples were obtained with NIH densitometry Image software and normalized.

RESULTS

The objective of this study was to utilize a focused gene discovery approach to identify genes whose expression is significantly altered in IDCM and to identify an expression pattern in female vs. male IDCM transplant recipients. We created both a forward (IDCM minus control) and a reverse (control minus IDCM) subtracted cDNA library and sequenced 1,000 randomly selected clones from each. Sequence clustering analysis produced 535 contigs (consensus sequence of clustered ESTs representing 1 gene) unique to the forward subtracted library (upregulated) and 495 contigs uniquely represented in the reverse subtracted library (downregulated). Sequences identified by means of BLAST alignment to the GenBank databases showed 95–100% homology at the nucleic acid level. Of the identified contigs, 75% had an assigned function.

The gene representation based on functional groups for each subtracted library is shown in Fig. 1. Genes of unknown function make up 29% and 22% of the forward and reverse subtracted libraries, respectively. Of the 157 contigs of unknown function found in the forward library, 133 are unique to the forward subtracted cDNA library (i.e., not found in the reverse subtracted cDNA library). Likewise, 84 of 108 contigs of unknown function are unique to the reverse subtracted cDNA library. As one might expect, a large proportion of genes (18%) associated with cellular growth and the extracellular matrix were identified in the forward library as opposed to only 4% in the reverse library. This expression pattern may be indicative of ventricular remodeling due to IDCM.

Table 3. *Differentially regulated genes in male IDCM patient samples*

Common Name	Description	Accession No.	Function
		Upregulated genes	
MFAP4	Microfibrillar-associated protein 4	NM_002404	Cell growth/ECM
LUM	Lumican	BC007038	Cell growth/ECM
ARGBP2	Arg/Abl-interacting protein	BC011883	Cytoskeleton
COL16A1	Type XVI collagen α-1 chain	S57132	Cell growth/ECM
DPT	Dermatopontin	NM_001937	Cell growth/ECM
CTGF	Connective tissue growth factor	NM_001901	Cell growth/ECM
MYH7	Myosin heavy chain polypeptide 7	NM_000257	Ca^{2+} binding & muscle contraction/structure
TTN	Titin	NM_003319	Ca^{2+} binding & muscle contraction/structure
FLJ14437	Myopalladin	NM_032578	Ca^{2+} binding & muscle contraction/structure
FSTL3	Follistatin-like 3	NM_005860	Cell growth/ECM
GPI	Glucose phosphate isomerase	NM_000175	Energetics & metabolism
HLA-DRB	Major histocompatibility complex class III	BC008403	Unknown
ACO2	Aconitase 2	BC014092	Energetics & metabolism
ANK1	Ankyrin 1	AF005213	Cell growth/ECM
LGMN	Legumain	NM_005606	Degradation
SLC4A3	Solute carrier family 4_anion exchanger member 3	NM_005070	Ion transport
HSPCB	Unknown	CN355913	Unknown
		Downregulated genes	
MYH6	Myosin heavy chain polypeptide 6	NM_002471	Ca^{2+} binding & muscle contraction/structure
PLA2G2A	Phospholipase A_2_group IIA	BC005919	Energetics & metabolism (lipid)
ADH1B	Alcohol dehydrogenase IB	NM_000668	Energetics & metabolism (alcohol)
MT1X	Metallothionein	BC032338	Metal ion binding
HRASLS3	HRAS-like suppressor 3	NM_007069	Unknown
PYGM	Highly similar to glycogen phosphorylase muscle form	AK056607	Energetics & metabolism (carbohydrate)
		R1229	Unknown
TIMP4	Tissue inhibitor of metalloproteinase 4	NM_003256	Cell growth/ECM
MGC29729	Hypothetical protein	BC032121	Unknown
MUSTN1	Musculoskeletal embryonic nuclear protein 1	NM_205853	Unknown
TRA1	Tumor rejection antigen	BC066656	Stress
G0S2	Putative lymphocyte G_0/G_1 switch gene	BC009694	Immune response
KCNK1	Potassium channel subfamily K-member 1	NM_002245	Ion transport
APOD	Apolipoprotein D	NM_001647	Energetics & metabolism (lipid)
IL13RA1	Interleukin 13 receptor α-1	NM_001560	Signal transduction

Genes up- or downregulated (>1.8-fold) in at least 3 of 5 male IDCM patient samples are shown.

Downloaded from journals.physiology.org/journal/physiolgenomics (073.004.114.249) on July 15, 2021.

To further explore IDCM-specific gene expression patterns, a human heart failure microarray was produced with our subtracted hybridization gene data. All contig sequences with both known and unknown function were used to produce an oligonucleotide-based human heart failure microarray. As a result, we created a focused heart failure gene array containing 1,145 heart-specific oligonucleotide probes.

To address the question of sex-specific gene expression in IDCM left ventricle tissue, individual IDCM RNA (female $n = 6$; male $n = 5$) were hybridized to our heart failure microarray against a male or female standard created from pooled samples of nonfailing female ($n = 10$) or male ($n = 10$) left ventricle RNA samples. Table 1 lists 55 genes determined to be differentially expressed in IDCM female patient samples (37 upregulated; 18 downregulated). Nineteen of those genes were found to be significantly upregulated (13 genes) or downregulated (6 genes) coordinately in the male patient samples, as depicted in Table 2. We used the same stringent criteria in males as in females for genes that were consistently and significantly deregulated in DCM patients (i.e., genes showing >1.8-fold up- or downregulation in 3 of 5 male and 3 of 6 female transplant recipients compared with the pooled male or female nonfailing control samples, respectively). Accordingly, Table 3 lists 30 genes determined to be differentially regulated in male IDCM patient samples (17 upregulated and 13 downregulated). No genes were found to be differentially regulated paradoxically (in opposite direction) between the male and female cohorts. Genes of known function were classified on the basis of biological function according to a modified version of the NCBI Gene Ontology (GO) classification scheme. The functional classification scheme consisted of 11 categories and subgroups within each category. Functional classifications within the expression clusters of female and male patient samples are illustrated in Fig. 2.

Comparison of differentially expressed genes in male and female IDCM samples based on functional category showed a female expression pattern. Genes encoding metabolic proteins made up a majority of the female IDCM upregulated expression pattern (28%). The majority of these genes are involved in oxidative phosphorylation (e.g., NADH dehydrogenase, malate dehydrogenase 1, and succinate dehydrogenase) and regulation of lipid and nucleic acid metabolism [e.g., acetyl-coenzyme A acetyltransferase poly(rC) binding protein 2]. Insulin-like growth factor binding protein 2 and latent transforming growth

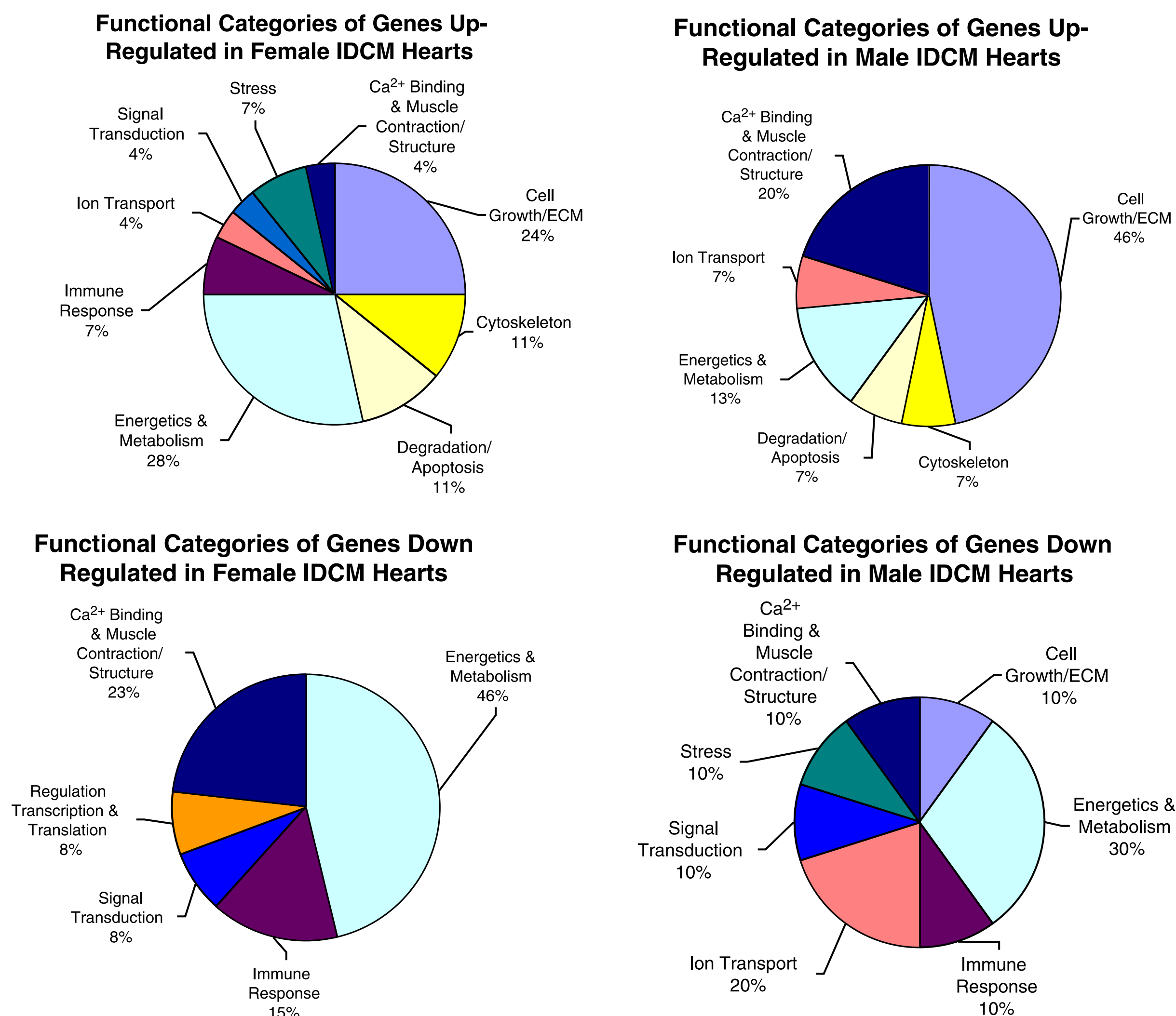

Fig. 2. Functional analysis of genes found to be differentially expressed in male and female IDCM left ventricle tissue by means of microarray analysis.

Downloaded from journals.physiology.org/journal/physiolgenomics (073.004.114.249) on July 15, 2021.

factor-β binding protein were uniquely upregulated only in the female cohort. Upregulation of these genes was not seen in the male cohort. Genes found to be upregulated in both male and female cohorts represented proteins involved in cell growth, cell adhesion, and the extracellular matrix. This observation is likely indicative of ventricular remodeling that occurs irrespective of sex. On the other hand, the male cohort showed a marked upregulation of genes related to calcium binding and muscular contraction compared with the female profile. In particular, the gene expressions of myosin heavy chain polypeptide 7, titin, and myopalladin were significantly elevated in male IDCM patients. In the same line, we also found fewer downregulations of gene expression related to calcium binding and contraction in male IDCM vs. female IDCM patients.

The most prevalent group of transcripts downregulated in both the male and female cohorts were those involved with lipid and carbohydrate metabolism. Apolipoprotein D and phospholipase A_2 were found to be coordinately downregulated in both the male and female cohorts. Likewise, glycogen phosphorylase (carbohydrate metabolism) was coordinately downregulated in both male and female cohorts, whereas glycerol-3-phosphate dehydrogenase 1 and NAD-dependent methylene tetrahydrofolate dehydrogenase cyclohydrolase (involved in 1-carbon metabolism) were uniquely downregulated only in the female cohort. Transcripts involved in calcium binding, muscle structure, and homeostasis including the S100 calcium binding protein A4, a LIM domain Ca^{2+} binding protein, and myosin light polypeptide 4 showed significantly lower levels of expression unique to the female data set.

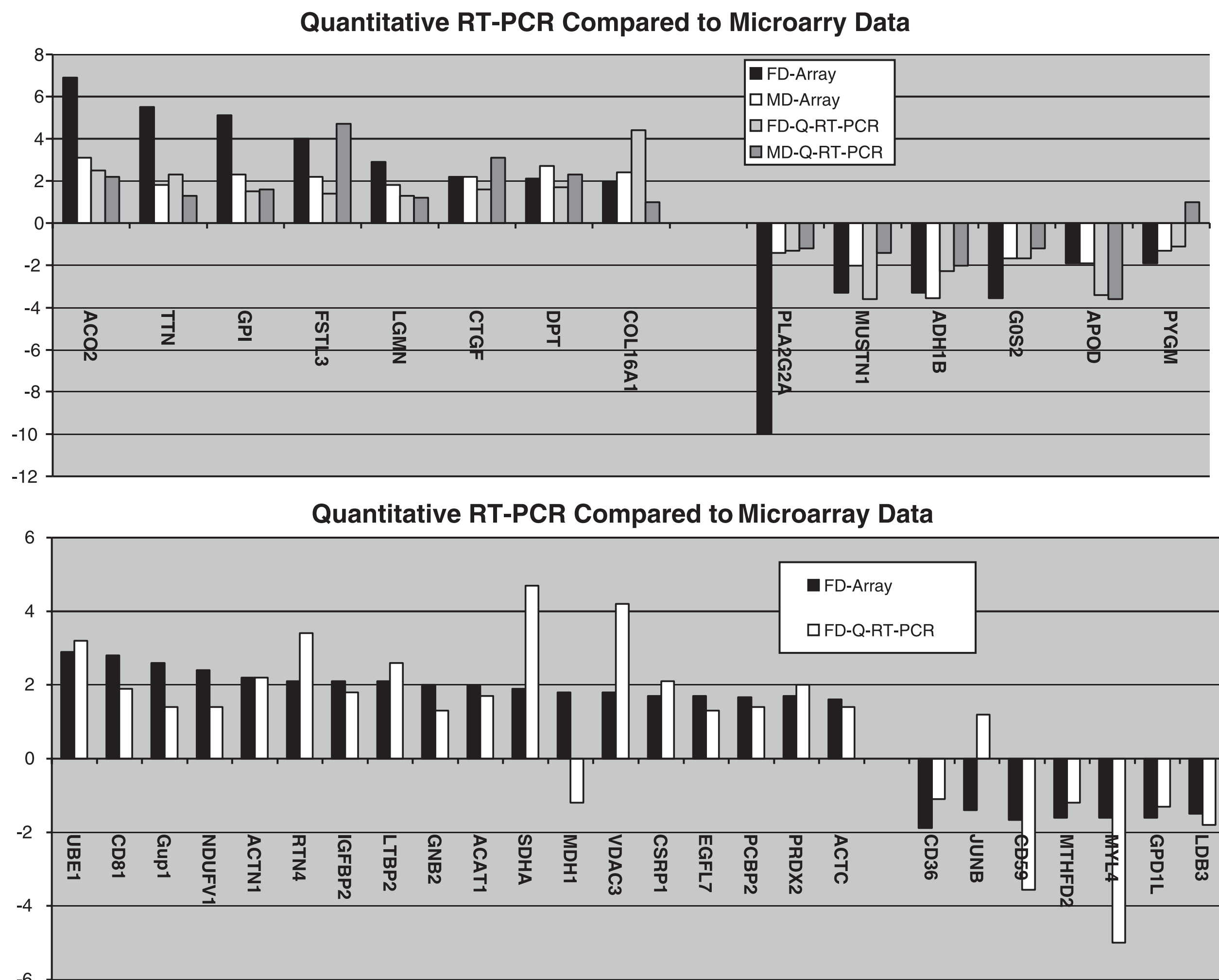

Fig. 3. Comparison of expression levels obtained by microarray analysis and by real-time quantitative RT-PCR (Q-RT-PCR) of 39 selected genes. Fold difference expression is represented for each pooled IDCM sample (female $n = 6$; male $n = 5$) relative to a pooled normal male or female control sample ($n = 20$). Positive numbers represent fold upregulated and negative numbers represent fold downregulated. Q-RT-PCR data were normalized to 18S rRNA. FD, female donor; MD, male donor; ACO2, aconitase 2; APOD, apolipoprotein D; COL16A1, collagen type XVI α1; CTGF, connective tissue growth factor; DPT, dermatopontin; G0S2, lymphocyte G_0/G_1 switch gene; GPI, glucose phosphate isomerase; MUSTN1, musculoskeletal embryonic nuclear protein 1; MYL4, myosin light polypeptide 4, alkali, atrial, embryonic; TTN, titin; ADH1B, alcohol dehydrogenase IB; FSTL3, myopalladin; LGMN, legumain; RTN4, reticulon 4; ACAT1, acetyl-coenzyme A acetyltransferase 1; Gup1, GRINL1A complex protein 2 precursor; LTBP2, latent transforming growth factor-β binding protein; MDH1, malate dehydrogenase 1, NAD (soluble); SDHA, succinate dehydrogenase complex, subunit A, flavoprotein; UBE1, ubiquitin-activating enzyme E1; ACTC, actin α, cardiac muscle; ACTN1, α-actinin; CD36, CD36 antigen; CD81, CD81 antigen; CSRP1, cysteine and glycine-rich protein 1; EGFL7, EGF-like-domain; GNB2, guanine nucleotide binding protein β polypeptide 2; GPD1L, glycerol-3-phosphate dehydrogenase 1-like; IGFBP2, insulin-like growth factor binding protein 2; LDB3, LIM domain binding 3; MTHFD2, NAD-dependent methylene tetrahydrofolate dehydrogenase cyclohydrolase; NDUFV1, NADH dehydrogenase (ubiquinone) flavoprotein 1; PCBP2, poly(rC) binding protein 2.

Downloaded from journals.physiology.org/journal/physiolgenomics (073.004.114.249) on July 15, 2021.

RT-PCR was used to confirm the relative expression patterns of 39 randomly selected transcripts identified as differentially expressed in IDCM by means of microarray analysis. Expression of 36 of the 39 genes (92.3%) tested by means of quantitative RT-PCR reflected results obtained by microarray analysis (see Fig. 3).

Furthermore, gene expression analysis of SERCA2a showed this gene to be downregulated in male human transplant recipients as assessed by microarray analysis. Western blot analysis (Fig. 4) corresponded to the male gene expression pattern and indicated an 88% decrease in protein content in the male human heart failure samples. However, microarray analysis of SERCA2a shows this gene to be upregulated in female human transplant recipients. Western blot analysis (Fig. 4) shows a 48% increase in the SERCA2a of human IDCM female hearts compared with the control hearts, which is in agreement with the gene modulation data.

DISCUSSION

IDCM is a common etiology of heart muscle disease in both male and female patients. Despite the fact that women suffering from IDCM often have clinical presentations and outcomes differing from those of men with a similar cardiac condition, little is known about the cellular or molecular basis of these sex-related differences. Since the structural and functional changes that occur in IDCM hearts are ultimately due to changes in gene and protein expression, we have explored the differences in myocardial male and female gene expression patterns consequent to IDCM. The use of subtractive hybridization to produce a focused heart failure array along with array hybridization of male and female myocardial RNA samples has identified genes whose expression is significantly altered in IDCM as well as an expression pattern unique to each sex transplant recipient with IDCM.

Microarray technology has been used as a large-scale genomic approach to decipher the molecular mechanisms involved in physiological and pathological processes in various animal or human tissues (1, 2, 5, 18, 19, 26, 30, 35, 56, 58). We have used our human heart failure array to further quantify the expression of gene products in female and male hearts consequent to IDCM. Each individual heart failure sample was normalized to sex-specific pooled nonfailing heart samples to standardize for normal differences in expression values between male and female samples. We selected our IDCM population, analyzing demographics as well as age matching our control human heart samples. Controls were selected from our human tissue repository that were the best matched on the basis of echocardiographic study.

Differences in the literature may also reflect differences in the techniques applied as well as data analysis approaches (14, 17, 25, 31, 33, 38, 43, 45, 48, 54, 65). We have applied a focused approach to gene expression analysis consequent to IDCM. We have created the first focused human heart failure microarray containing 1,145 differentially expressed gene probes. A random sample of genes demonstrated by microarray to be deregulated was confirmed by RT-PCR with 92.3% conformity. Using this stringent approach, we have identified sex-specific gene expression patterns in female and male transplant recipients with IDCM.

At the outset of this study, we anticipated that some of the results would confirm the accuracy of our array based on previous published observations. For example, natriuretic peptide precursor A (NPPA) showed a high level of upregulation in IDCM transplant recipients (i.e., male and female), supporting its role as a marker of myocardial stress (67). SERCA2a was consistently downregulated in male samples and tended to be upregulated in female samples. Western blot analysis demonstrated an 88% decrease in protein content in male IDCM samples compared with male control samples. In contrast, a 48% increase in SERCA2a protein content was observed in female IDCM samples compared with control female samples. This gene product is being targeted for molecular medicine approaches and may warrant closer scrutiny. The high stringency of the microarray analysis would exclude certain genes that otherwise would show deregulation with a less stringent analysis or Western blot assays. Thus the outset is affected by the threshold for inclusion, which depends on the stringency level predefined before analysis.

In addition to genes whose role in IDCM is well characterized, data were obtained identifying genes and families of genes whose correlation with disease has not yet been reported. Not surprisingly, there was a significant overlap of upregulated genes (13 of 37 genes upregulated in female IDCM) between male and female IDCM patients. Most of the genes coordinately upregulated in both the male and female groups were those involved in general cell growth and the extracellular matrix, indicative of myocyte and ventricular remodeling common to both. The largest group of genes consistently upregulated in IDCM female heart samples were those involved in energy and metabolism (e.g., mitochondrial oxidative phosphorylation and ATP synthesis). DCM involves structural as well as functional alterations leading to heart failure. Metabolic cardiac stress is known to be associated with the development of DCM, ultimately leading to an imbalance and heart failure. Therefore, the differential gene regulation between sexes may be reflected in a difference in the tolerance levels for the disease between males and females.

In the stressed heart, metabolic remodeling precedes, triggers, and sustains functional and structural remodeling (42, 51, 55, 59, 64). Adaptations to sustained heart stress can induce changes in the metabolic machinery at a transcriptional and/or a translational level of enzymes involved in metabolic pathways. Concomitant with an increase of gene expression of en-

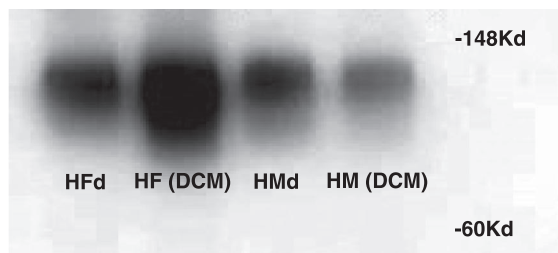

Fig. 4. Western blot of sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) of human male and female cardiomyocytes. Two hundred micrograms of total protein was used for each sample. Relative specific protein levels between IDCM and nonfailing donor samples were obtained with NIH densitometry software (NIH Image). Human male donor (HMd) and human female donor (HFd) = 200 μg of total protein isolated from human male donor left ventricle tissue (normal). Human male IDCM [HM(DCM)] and human female IDCM [HF(DCM)] = 200 μg of total protein isolated from human male patients undergoing cardiac transplantation (IDCM).

Downloaded from journals.physiology.org/journal/physiolgenomics (073.004.114.249) on July 15, 2021.

zymes associated with oxidative phosphorylation was an increase in peroxiredoxin 2 in female-specific analysis, an enzyme involved in the reduction of the oxygen radical hydrogen peroxide (a destructive by-product of oxidative phosphorylation). This observation of deregulation of genes associated with energy transduction and antioxidant activity unique to female patients may suggest a higher level of metabolic adaptation in female hearts due to stress, resulting in increased myocyte survival. In fact, a proteomic study has shown that, in contrast to aging male monkey hearts, hearts of female nonhuman primates did not undergo reductions in the expression of enzymes related to glycolysis, glucose oxidation, TCA cycle and mitochondrial oxidative phosphorylation (69). This supports our data showing a higher metabolic capacity and energy production of female hearts compared with male hearts, conferring a better survival than males. More recently, the same group, using DNA microarray, showed an upregulation of phosphor-transferase genes in female monkeys, which are related to glycolysis and glucose oxidation (53). In that regard, the cDNA array data from our female cohort show that a major deregulated gene group is related to metabolic disorders. Specifically, those that are related to glycolysis are downregulated, while those related to oxidative phosphorylation are upregulated. Such deregulation is specific to the female patients, since the male patients did not exhibit it. Looking at the broader picture regarding the signaling pathways, it seems that female patients with IDCM have a downgraded glycolysis, so that the source of energy production (ATP) may be shunted from glycolysis toward other collateral pathways that feed into the upregulated TCA or oxidative phosphorylation process. Such inputs could be from fatty acid metabolism, ketone bodies, amino acids, and their metabolites (e.g., creatine). Accordingly, cardiomyopathy has been associated with mitochondrial disease (32). It has been suggested that the supply side of heart failure (i.e., myocardial energetics) is the most plastic and easily restored (36, 52, 68). This might in part explain the somewhat better outcomes for women with heart failure. We previously demonstrated (27, 46) in a model of heart failure that rescuing the normal phenotype is correlated with a return toward normal myocardial energetics, sarcoplasmic reticulum calcium cycling, and myofibrillar ATPase. This distinctive better tolerance to failure exhibited by female IDCM vs. male IDCM patients is congruent with larger upregulation of calcium binding and fewer downregulations of muscle contraction gene expressions in our male patients. This may be regarded as an attempt to improve systolic function of the dilated heart in the male, but it may also prove to be exhaustive of the cardiac muscle's energetics in the long term. Such energy burden is aggravated with the large downregulation of gene expressions related to energetics and metabolism in the male.

The link between abnormal heart metabolism and progression to heart failure has been well studied (27, 36, 42, 46, 51, 52, 55, 63, 64). It has been suggested that energy supply and demand are pivotal in the development and progression of heart failure (36, 42, 59, 64). There is striking evidence in cancer cells that implicates a link between cell survival and metabolism. Cancer cells have been shown to possess an increased rate of glucose metabolism and oxidative phosphorylation accompanied by a reduction in cell death when stressed (29, 68). Perhaps female hearts possess a greater ability, due to heightened metabolic adaptation, to maintain energy for contractile function in a stressed heart, leading to less cardiac dysfunction, cell death, and remodeling. The modulation of energy metabolism to improve performance of dysfunctional myocardium has been intensely studied (16, 55, 60). Pharmacological agents that increase myocardial substrate metabolism and further couple this process with oxidative phosphorylation have been shown to have beneficial effects in heart failure (9, 13, 16, 40, 42, 55, 57, 60). These data suggest that increased compensatory mechanisms in female heart failure may lie in increased or prolonged efficiency of metabolic adaptation. Further study is needed to assess any increased beneficial effects of metabolic modulation in female heart failure that may improve metabolic/mechanical coupling.

In conclusion, we have identified molecular pathways that may mediate sex differences in IDCM prognosis. One can envision the future use of expression data in clinical trial enrollments. Sex-specific differences in the expression profile of critical genes could provide important indicators and diagnostic markers of heart failure progression in women as well as men. Expression studies such as this one could serve to identify genes encoding proteins that are drug target candidates in female as well as male heart failure patients. In this study we focused on end-stage IDCM. This may not reflect early stages in diseased myocardium. Future exploration of gene expression patterns in early states of diseased myocardium is warranted. Ongoing work also includes race-related profiling of gene expression levels.

GRANTS

This work is supported by National Institutes of Health Grants R43-HL-67516, R44-HL-67516, R43-AA-066758, and R44-AA-066758 to J. K. Gwathmey.

REFERENCES

1. **Aach J, Rindone W, Church GM.** Systematic management and analysis of yeast gene expression data. *Genome Res* 10: 431–445, 2000.
2. **Abraham WT, Gilbert EM, Lowes BD, Minobe WA, Larrabee P, Roden RL, Dutcher D, Sederberg J, Lindenfeld JA, Wolfel EE, Shakar SF, Ferguson D, Volkman K, Linseman JV, Quaife RA, Robertson AD, Bristow MR.** Coordinate changes in myosin heavy chain isoform gene expression are selectively associated with alterations in dilated cardiomyopathy phenotype. *Mol Med* 8: 750–760, 2002.
3. **Adams KF Jr, Sueta CA, Gheorghiade M, O'Connor CM, Schwartz TA, Koch GG, Uretsky B, Swedberg K, McKenna W, Soler-Soler J, Califf RM.** Gender differences in survival in advanced heart failure. Insights from the FIRST study. *Circulation* 99: 1816–1821, 1999.
4. **American Heart Association.** *Heart Disease and Stroke Statistics—2005 Update*. Dallas, TX: American Heart Association, 2005.
5. **Barrans JD, Allen PD, Stamatiou D, Dzau VJ, Liew CC.** Global gene expression profiling of end-stage dilated cardiomyopathy using a human cardiovascular-based cDNA microarray. *Am J Pathol* 160: 2035–2043, 2002.
6. **Barth AS, Kuner R, Buness A, Ruschhaupt M, Merk S, Zwermann L, Kaab S, Kreuzer E, Steinbeck G, Mansmann U, Poustka A, Nabauer M, Sultmann H.** Identification of a common gene expression signature in dilated cardiomyopathy across independent microarray studies. *J Am Coll Cardiol* 48: 1610–1617, 2006.
7. **Barth AS, Merk S, Arnoldi E, Zwermann L, Kloos P, Gebauer M, Steinmeyer K, Bleich M, Kaab S, Pfeufer A, Uberfuhr P, Dugas M, Steinbeck G, Nabauer M.** Functional profiling of human atrial and ventricular gene expression. *Pflügers Arch* 450: 201–208, 2005.
8. **Beisvag V, Lehre PK, Midelfart H, Aass H, Geiran O, Sandvik AK, Laegreid A, Komorowski J, Ellingsen O.** Aetiology-specific patterns in end-stage heart failure patients identified by functional annotation and classification of microarray data. *Eur J Heart Fail* 8: 381–389, 2006.
9. **Bersin RM, Wolfe C, Kwasman M, Lau D, Klinski C, Tanaka K, Khorrami P, Henderson GN, de Marco T, Chatterjee K.** Improved hemodynamic function and mechanical efficiency in congestive heart

Downloaded from journals.physiology.org/journal/physiolgenomics (073.004.114.249) on July 15, 2021.

failure with sodium dichloroacetate. *J Am Coll Cardiol* 23: 1617–1624, 1994.
10. **Blackman IC, Bond M, Bowling A, Banning A, Dudley N, Elder A, Martin A, Rai GS.** Age and sex do not bias the use of angiotensin-converting enzyme inhibitors in acute myocardial infarction and congestive heart failure. *J Am Geriatr Soc* 51: 572–573, 2003.
11. **Boheler KR, Volkova M, Morrell C, Garg R, Zhu Y, Margulies K, Seymour AM, Lakatta EG.** Sex- and age-dependent human transcriptome variability: implications for chronic heart failure. *Proc Natl Acad Sci USA* 100: 2754–2759, 2003.
12. **Bozdech Z, Zhu J, Joachimiak MP, Cohen FE, Pulliam B, DeRisi JL.** Expression profiling of the schizont and trophozoite stages of *Plasmodium falciparum* with a long-oligonucleotide microarray. *Genome Biol* 4: R9, 2003.
13. **Bricaud H, Brottier L, Barat JL, Combe C, Boussens B, Bonnet J.** Cardioprotective effect of trimetazidine in severe ischemic cardiomyopathy. *Cardiovasc Drugs Ther* 4, *Suppl* 4: 861–865, 1990.
14. **Broude NE.** Differential display in the time of microarrays. *Expert Rev Mol Diagn* 2: 209–216, 2002.
15. **Carroll JD, Carroll EP, Feldman T, Ward DM, Lang RM, McGaughey D, Karp RB.** Sex-associated differences in left ventricular function in aortic stenosis of the elderly. *Circulation* 86: 1099–1107, 1992.
16. **Chapados RA, Gruver EJ, Ingwall JS, Marsh JD, Gwathmey JK.** Chronic administration of cardiovascular drugs: altered energetics and transmembrane signaling. *Am J Physiol Heart Circ Physiol* 263: H1576–H1586, 1992.
17. **Churchill GA.** Fundamentals of experimental design for cDNA microarrays. *Nat Genet* 32, *Suppl*: 490–495, 2002.
18. **Clark EA, Golub TR, Lander ES, Hynes RO.** Genomic analysis of metastasis reveals an essential role for RhoC. *Nature* 406: 532–535, 2000.
19. **Coller HA, Grandori C, Tamayo P, Colbert T, Lander ES, Eisenman RN, Golub TR.** Expression analysis with oligonucleotide microarrays reveals that MYC regulates genes involved in growth, cell cycle, signaling, and adhesion. *Proc Natl Acad Sci USA* 97: 3260–3265, 2000.
20. **Du XJ.** Gender modulates cardiac phenotype development in genetically modified mice. *Cardiovasc Res* 63: 510–519, 2004.
21. **Du XJ, Samuel CS, Gao XM, Zhao L, Parry LJ, Tregear GW.** Increased myocardial collagen and ventricular diastolic dysfunction in relaxin deficient mice: a gender-specific phenotype. *Cardiovasc Res* 57: 395–404, 2003.
22. **Ellinghaus P, Scheubel RJ, Dobrev D, Ravens U, Holtz J, Huetter J, Nielsch U, Morawietz H.** Comparing the global mRNA expression profile of human atrial and ventricular myocardium with high-density oligonucleotide arrays. *J Thorac Cardiovasc Surg* 129: 1383–1390, 2005.
23. **Gao XM, Agrotis A, Autelitano DJ, Percy E, Woodcock EA, Jennings GL, Dart AM, Du XJ.** Sex hormones and cardiomyopathic phenotype induced by cardiac beta 2-adrenergic receptor overexpression. *Endocrinology* 144: 4097–4105, 2003.
24. **Gao Z, Xu H, DiSilvestre D, Halperin VL, Tunin R, Tian Y, Yu W, Winslow RL, Tomaselli GF.** Transcriptomic profiling of the canine tachycardia-induced heart failure model: global comparison to human and murine heart failure. *J Mol Cell Cardiol* 40: 76–86, 2006.
25. **Gerhold DL, Jensen RV, Gullans SR.** Better therapeutics through microarrays. *Nat Genet* 32, *Suppl*: 547–551, 2002.
26. **Grzeskowiak R, Witt H, Drungowski M, Thermann R, Hennig S, Perrot A, Osterziel KJ, Klingbiel D, Scheid S, Spang R, Lehrach H, Ruiz P.** Expression profiling of human idiopathic dilated cardiomyopathy. *Cardiovasc Res* 59: 400–411, 2003.
27. **Gwathmey JK, Kim CS, Hajjar RJ, Khan F, DiSalvo TG, Matsumori A, Bristow MR.** Cellular and molecular remodeling in a heart failure model treated with the beta-blocker carteolol. *Am J Physiol Heart Circ Physiol* 276: H1678–H1690, 1999.
28. **Haghighi K, Schmidt AG, Hoit BD, Brittsan AG, Yatani A, Lester JW, Zhai J, Kimura Y, Dorn GW, MacLennan DH, Kranias EG.** Superinhibition of sarcoplasmic reticulum function by phospholamban induces cardiac contractile failure. *J Biol Chem* 276: 24145–24152, 2001.
29. **Hanahan D, Weinberg RA.** The hallmarks of cancer. *Cell* 100: 57–70, 2000.
30. **Harrington CA, Rosenow C, Retief J.** Monitoring gene expression using DNA microarrays. *Curr Opin Microbiol* 3: 285–291, 2000.
31. **Hess KR, Zhang W, Baggerly KA, Stivers DN, Coombes KR.** Microarrays: handling the deluge of data and extracting reliable information. *Trends Biotechnol* 19: 463–468, 2001.
32. **Holmgren D, Wahlander H, Eriksson BO, Oldfors A, Holme E, Tulinius M.** Cardiomyopathy in children with mitochondrial disease; clinical course and cardiological findings. *Eur Heart J* 24: 280–288, 2003.
33. **Holzman T, Kolker E.** Statistical analysis of global gene expression data: some practical considerations. *Curr Opin Biotechnol* 15: 52–57, 2004.
34. **Horn S, Lueking A, Murphy D, Staudt A, Gutjahr C, Schulte K, Konig A, Landsberger M, Lehrach H, Felix SB, Cahill DJ.** Profiling humoral autoimmune repertoire of dilated cardiomyopathy (DCM) patients and development of a disease-associated protein chip. *Proteomics* 6: 605–613, 2006.
35. **Hwang JJ, Allen PD, Tseng GC, Lam CW, Fananapazir L, Dzau VJ, Liew CC.** Microarray gene expression profiles in dilated and hypertrophic cardiomyopathic end-stage heart failure. *Physiol Genomics* 10: 31–44, 2002.
36. **Ingwall JS, Nascimben L, Gwathmey JK.** Heart failure: is the pathology due to calcium overload or to mismatch in energy supply and demand? In: *Heart Failure: Basic Science and Clinical Aspects*, edited by Gwathmey JK, Briggs GM, and Allen PD. New York: Dekker, 1993, p. 667–700.
37. **Kadokami T, McTiernan CF, Kubota T, Frye CS, Feldman AM.** Sex-related survival differences in murine cardiomyopathy are associated with differences in TNF-receptor expression. *J Clin Invest* 106: 589–597, 2000.
38. **Kaminski N, Allard JD, Pittet JF, Zuo F, Griffiths MJ, Morris D, Huang X, Sheppard D, Heller RA.** Global analysis of gene expression in pulmonary fibrosis reveals distinct programs regulating lung inflammation and fibrosis. *Proc Natl Acad Sci USA* 97: 1778–1783, 2000.
39. **Kittleson MM, Minhas KM, Irizarry RA, Ye SQ, Edness G, Breton E, Conte JV, Tomaselli G, Garcia JG, Hare JM.** Gene expression analysis of ischemic and nonischemic cardiomyopathy: shared and distinct genes in the development of heart failure. *Physiol Genomics* 21: 299–307, 2005.
40. **Kobayashi A, Masumura Y, Yamazaki N.** L-Carnitine treatment for congestive heart failure—experimental and clinical study. *Jpn Circ J* 56: 86–94, 1992.
41. **Lashkari DA, DeRisi JL, McCusker JH, Namath AF, Gentile C, Hwang SY, Brown PO, Davis RW.** Yeast microarrays for genome wide parallel genetic and gene expression analysis. *Proc Natl Acad Sci USA* 94: 13057–13062, 1997.
42. **Lehman JJ, Kelly DP.** Gene regulatory mechanisms governing energy metabolism during cardiac hypertrophic growth. *Heart Fail Rev* 7: 175–185, 2002.
43. **Lemon WJ, Palatini JJ, Krahe R, Wright FA.** Theoretical and experimental comparisons of gene expression indexes for oligonucleotide arrays. *Bioinformatics* 18: 1470–1476, 2002.
44. **Li Y, Kishimoto I, Saito Y, Harada M, Kuwahara K, Izumi T, Hamanaka I, Takahashi N, Kawakami R, Tanimoto K, Nakagawa Y, Nakanishi M, Adachi Y, Garbers DL, Fukamizu A, Nakao K.** Androgen contributes to gender-related cardiac hypertrophy and fibrosis in mice lacking the gene encoding guanylyl cyclase-A. *Endocrinology* 145: 951–958, 2004.
45. **Liang P.** A decade of differential display. *Biotechniques* 33: 338–44, 346, 2002.
46. **Liao R, Nascimben L, Friedrich J, Gwathmey JK, Ingwall JS.** Decreased energy reserve in an animal model of dilated cardiomyopathy. Relationship to contractile performance. *Circ Res* 78: 893–902, 1996.
47. **Liew CC.** Expressed genome molecular signatures of heart failure. *Clin Chem Lab Med* 43: 462–469, 2005.
48. **Livesey FJ.** Strategies for microarray analysis of limiting amounts of RNA. *Brief Funct Genomic Proteomic* 2: 31–36, 2003.
49. **Margulies KB, Matiwala S, Cornejo C, Olsen H, Craven WA, Bednarik D.** Mixed messages: transcription patterns in failing and recovering human myocardium. *Circ Res* 96: 592–599, 2005.
50. **Marsh JD, Lehmann MH, Ritchie RH, Gwathmey JK, Green GE, Schiebinger RJ.** Androgen receptors mediate hypertrophy in cardiac myocytes. *Circulation* 98: 256–261, 1998.
51. **Neubauer S, Horn M, Naumann A, Tian R, Hu K, Laser M, Friedrich J, Gaudron P, Schnackerz K, Ingwall JS.** Impairment of energy metabolism in intact residual myocardium of rat hearts with chronic myocardial infarction. *J Clin Invest* 95: 1092–1100, 1995.
52. **O'Brien PJ, Gwathmey JK.** Myocardial Ca^{2+}- and ATP-cycling imbalances in end-stage dilated and ischemic cardiomyopathies. *Cardiovasc Res* 30: 394–404, 1995.

Downloaded from journals.physiology.org/journal/physiolgenomics (073.004.114.249) on July 15, 2021.

53. **Qiu H, Tian B, Resuello RG, Natividad FF, Peppas A, Shen YT, Vatner DE, Vatner SF, Depre C.** Sex-specific regulation of gene expression in the aging monkey aorta. *Physiol Genomics* 29: 169–180, 2007.
54. **Rao JS, Bond M.** Microarrays: managing the data deluge. *Circ Res* 88: 1226–1227, 2001.
55. **Saupe KW, Spindler M, Tian R, Ingwall JS.** Impaired cardiac energetics in mice lacking muscle-specific isoenzymes of creatine kinase. *Circ Res* 82: 898–907, 1998.
56. **Sharma HS, Peters TH, Moorhouse MJ, van der Spek PJ, Bogers AJ.** DNA microarray analysis for human congenital heart disease. *Cell Biochem Biophys* 44: 1–9, 2006.
57. **Stanley WC, Lopaschuk GD, Hall JL, McCormack JG.** Regulation of myocardial carbohydrate metabolism under normal and ischaemic conditions. Potential for pharmacological interventions. *Cardiovasc Res* 33: 243–257, 1997.
58. **Steenman M, Chen YW, Le CM, Lamirault G, Varro A, Hoffman E, Leger JJ.** Transcriptomal analysis of failing and nonfailing human hearts. *Physiol Genomics* 12: 97–112, 2003.
59. **Taegtmeyer H.** Genetics of energetics: transcriptional responses in cardiac metabolism. *Ann Biomed Eng* 28: 871–876, 2000.
60. **Taegtmeyer H, Wilson CR, Razeghi P, Sharma S.** Metabolic energetics and genetics in the heart. *Ann NY Acad Sci* 1047: 208–218, 2005.
61. **Tamura T, Said S, Gerdes AM.** Gender-related differences in myocyte remodeling in progression to heart failure. *Hypertension* 33: 676–680, 1999.
62. **Tan FL, Moravec CS, Li J, Apperson-Hansen C, McCarthy PM, Young JB, Bond M.** The gene expression fingerprint of human heart failure. *Proc Natl Acad Sci USA* 99: 11387–11392, 2002.
63. **Tian R.** Transcriptional regulation of energy substrate metabolism in normal and hypertrophied heart. *Curr Hypertens Rep* 5: 454–458, 2003.
64. **Tian R, Nascimben L, Kaddurah-Daouk R, Ingwall JS.** Depletion of energy reserve via the creatine kinase reaction during the evolution of heart failure in cardiomyopathic hamsters. *J Mol Cell Cardiol* 28: 755–765, 1996.
65. **Tilstone C.** DNA microarrays: vital statistics. *Nature* 424: 610–612, 2003.
66. **van Eickels M, Grohe C, Cleutjens JP, Janssen BJ, Wellens HJ, Doevendans PA.** 17β-Estradiol attenuates the development of pressure-overload hypertrophy. *Circulation* 104: 1419–1423, 2001.
67. **Vikstrom KL, Bohlmeyer T, Factor SM, Leinwand LA.** Hypertrophy, pathology, and molecular markers of cardiac pathogenesis. *Circ Res* 82: 773–778, 1998.
68. **Warburg O.** On the origin of cancer cells. *Science* 123: 309–314, 1956.
69. **Yan L, Ge H, Li H, Lieber SC, Natividad F, Resuello RR, Kim SJ, Akeju S, Sun A, Loo K, Peppas AP, Rossi F, Lewandowski ED, Thomas AP, Vatner SF, Vatner DE.** Gender-specific proteomic alterations in glycolytic and mitochondrial pathways in aging monkey hearts. *J Mol Cell Cardiol* 37: 921–929, 2004.
70. **Yang J, Moravec CS, Sussman MA, DiPaola NR, Fu D, Hawthorn L, Mitchell CA, Young JB, Francis GS, McCarthy PM, Bond M.** Decreased SLIM1 expression and increased gelsolin expression in failing human hearts measured by high-density oligonucleotide arrays. *Circulation* 102: 3046–3052, 2000.
71. **Yoshioka S, Takahashi Y, Okimura Y, Takahashi K, Iguchi G, Iida K, Kaji H, Chihara K.** Gene expression profile in the heart of spontaneous dwarf rat: in vivo effects of growth hormone. *Biochem Biophys Res Commun* 341: 88–93, 2006.

Downloaded from journals.physiology.org/journal/physiolgenomics (073.004.114.249) on July 15, 2021.