SUPPLEMENTAL MATERIAL

Measurement of Left Ventricular Volume and Ejection Fraction by Radionuclide Techniques and Echocardiography

Radionuclide ventriculography was performed at baseline, 3, and 12 months. Patient blood samples were labeled with $^{99m}TcO_4$, using the Ultratag (Mallinckrodt Pharmaceuticals, Dublin, Ireland) labeling technique. Labeled red blood cells were re-injected during dynamic first pass image acquisition to obtain right ventricular ejection fraction. Gated tomographic single-photon emission computed tomographic (SPECT) imaging was performed (Prism 3000, Picker International, Cleveland OH or dual head Siemens E-CAM, Hoffman Estates, IL) and analyzed using a 4DMPECT angiographic analysis package (INVIA, Ann Arbor MI). When SPECT could not be performed, LVEF was determined by clinical multi-gated acquisition. As a secondary indicator of LV remodeling and for screening purposes, left ventricular internal systolic and diastolic dimensions were measured at the mitral valve leaflet tips in the parasternal short axis view using M-mode echocardiography in accordance with American Society of Echocardiography recommendations.

RNA Extraction from Endomyocardial Biopsy Material

Biopsy specimens were immediately frozen in liquid nitrogen and stored at -80°C. All specimens from the same patient were processed together.{Lowes 2002} RNA was extracted from 10-15 mg of tissue using a motorized homogenizer and a guanidinium thiocyanate-phenol-chloroform reagent followed by affinity column-based purification and enzyme-based genomic DNA/protein removal. RNA mass was calculated from the absorbance at 260 nm (A260) using spectrophotometry,

and purity was inferred from the A260:A280 ratio. Purified RNA was aliquoted and stored at -80° C.

Reverse Transcription-Quantitative Polymerase Chain Reaction

First-strand cDNA was synthesized from 4.0 μg of total RNA using random primers (High Capacity Reverse Transcription Kit, ABI). Resultant cDNA pools were aliquoted for TaqMan fluorescent RT-qPCR and stored at -80° C. Assays for all candidate genes were run on an ABI 7300 in 96-well plates. All samples for each patient were assayed on the same plate for any individual gene, and each plate included *GAPDH* and *18s rRNA* as reference controls. Primers were designed using ABI Primer Express Software (v. 1.0 and 3.0, see Supplement) and either tested for amplification efficiency on an ABI 7300 or ordered as Pre-Designed Assays (reference control genes). Data files were analyzed using ABI 7300 Software (v. 2.0.6) to verify baseline and threshold cycle (C_t) detection.

mRNA Measurements by Microarray

cDNAs were subjected to chemical/enzymatic fragmentation (FL-Ovation® cDNA Biotin Module V2, NuGEN Technologies). Single–stranded 50-100 nucleotide fragments were labeled with a 3' biotin-labeled nucleotide, and approximately 3.75 μg of nucleotide fragments were hybridized to the Affymetrix HG-U133 Plus 2.0 Human Expression GeneChip after dilution into 200 μL of Affymetrix Hybridization Cocktail (Hybridization Buffer, DMSO and control oligonucleotides) for 16-20 hours

at 45˚C. The GeneChip was washed, stained with streptavidin-phycoerythrin, and read at a resolution of 6 microns with a Hewlett-Packard Gene Array Scanner.

RT-qPCR primers for candidate (n=50) and reference (n=2) genes

Symbol	Gene Name	Forward Primer	Reverse Primer	FAM Probe
ADRB1	Adrenergic receptor β1	CTTCATCATGTCCCTGGC	AACGGCACCACCAGCA	AGCGCCGACCTGGTCATGG
ADRB2	Adrenergic receptor β2	AGCCCTCAAGACGTTAGG	AGGTTATCCTGGATCACATG	ATCATCATGGGCACTTTCACCCT
HNRNPD	Heterogeneous nuclear ribonucleoprotein D (AUF-1)	GCGAAGATTGACGCCAGTAAG	TCTTTCTTTGTAGTGTCCCAGCTAAG	ACGAGGAGGATGAAGGGAAAATGTTTATAGGAGG
ADRBK1	beta adrenergic receptor kinase 1 (BARK1, GRK2)	CTTCGTGGTCTACCGGGAC	CGCACGTGGCCATGC	AAGCCAGCCAACATCCTTCTGGACG
GNAS	GNAS complex locus (alpha subunit Gs)	GCGATGAACGCCGCA	GCCACCACGAAGATGATGG	TGGATCCAGTGCTTCAACGATGTGACT
GNAI2	G protein, α inhibiting activity polypeptide 2 ($G_{\alpha i^{-}\#}$)	CGCCGTCACCGATGTCA	AAAGTCGGCGGTGGCC	CATCAAGAACAACCTGAAGGACTGCGG
ADCY5	Adenylate cyclase 5	GGAGGCTGGCGGCAA	GTCCCCATTCAGGTAGTTGAGTG	AGGACGCATCCACATCACCAAGGCT
ADRA1A	Adrenergic receptor α 1A	GCTGGCTGCCTTTTTTCTTAGTC	AAAAACTGTTTCAGAGGGCTTGA	TGCCCATTGGGTCTTTCTTCCCTGAT
GNAQ	G protein, q polypeptide ($G_{\alpha\theta}$)	CCCCACCACAGGGATCATC	TTTGGCCCCCTACATCGA	AATACCCCTTTGACTTACAAAGTGTCATTTTCAGAATG
PRKCB	Protein kinase C, β	TCGGGAAGCAGGGATTCC	AAATTCATGGCACCGCTTG	TGCCAAGTTTGCTGCTTTGTGGTGC
SLC9A1	Solute carrier family 9, (sodium/hydrogen exchanger), member 1	TCCCTAGATCCAGGCTTCTC	GATTGGTCTGAATGAGGAGGA	ACCTGGACTAGGGCCTCGGAGG
ACE	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	GCAGTACAACTGGACGCCGAAC	GGCCTGCTGCGCATCC	CCGCTCGCTCAGAAGGGC
AGT	Angiotensinogen	CTCAACTGGATGAAGAAACTGTCT	CATAAGATCCTTGCAGCACC	CCGGACCATCCACCTGACCATG
AGTR1	Angiotensin II receptor, type 1	AAATGGCTGGGTTTTTATCTGAATA	TTTTGATCACCTGGGTCGAATT	ATGCCATCCCAGAAAGTCGGCACC
EDN1	Endothelin 1	ACCTGGACATCATTTGGGTCA	TGGACCTAGGGCTTCCAAGTC	CACTCCCGAGCACGTTGTTCCGT
HK2	Hexokinase 2	GGCTCAAGACAAGGGGCA	GATGGCTCGGACTTGCAG	AAGTTCTTGTCTCAGATTGAGAGTGACTGCCTG
PFKM	Phosphofructokinase, muscle	GAGGGCTCTGGTCTTCCAAC	TGTTCCTTGGGGATTCGATG	CTGAGCTGAAGGACCAGACAGATTTTGA
PDK4	Pyruvate dehydrogenase kinase, isozyme 4	TCCACTGCACCAACGCCT	GCAAGCCGTAACCAAAACCA	ATGGATAATTCCCGGAATGCTCCTTTGG
PDHX	Pyruvate dehydrogenase complex, component X	AGTTAGGCAAGATCTGGTCAAAG	CCTCTCCATCCCAGCTTACA	CAGCTGTTACCCTTAAACAAATGCCAGATGT
CPT1B	Carnitine palmitoyltransferase 1B	CATTGCTGATCTTTTCCAAG	GGGCAGCTGGCATTT	CCCAAGGCCTACAGCTGAAGGTTG
ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 (Serca 2a)	CCAGTGGCTGATGGTGCT	ACTTGAGCGTCTCATCCATG	AAAATCTCCTTGCCCGTGATTC
PLN	Phospholamban	CACAATACTCTATACTGTGATGATCACAG	AAAGCTGGCAGCCAAATATG	TGCCAAGGCTACCTAAAAGAAGACAGT
RYR2	Ryanodine receptor 2 (cardiac)	TTCTTCTGCATCATTGGATACTACT	TTCCGTGCCACTTCCTTT	TTGAAAGTCCCATTGGTTATTTTTAAGCGA
CASQ2	Calsequestrin 2 (cardiac muscle)	*Life Technologies (Carlsbad, CA) TaqMan Gene Expression Assay : Hs00904422_m1		
SLC8A1	Solute carrier family 8 (sodium/calcium exchanger), member 1	GCTGCACCATTGGCCTGA	CTGCCACTTTGCTGGCAAA	ACTGCAGTCGTGTTCGTCGCACTTG
CANX	Calnexin	*Life Technologies (Carlsbad, CA) TaqMan Gene Expression Assay : Hs00233492_m1		
MYH6	Myosin, heavy chain 6, cardiac muscle, alpha	TCGCTGAGTCCCAGGTCA	TCCTCATCGTGCATTTTTTG	CCAAGAGCCGTGACATTGGTGCCA
MYH7	Myosin, heavy chain 7, cardiac muscle, beta	TGACATTGGCACGAAGG	AGCTGTTACACAGGCTCCAG	CCTGGAGGTGCCAGCAAAGC
ACTA1	actin, α1, skeletal muscle	GGCACCCAGCACCATGA	CCACACCGAGTATTTGCGCT	ATCAAGATCATCGCCCCGCCG
ACTC1	Actin, α1, cardiac muscle	CATCCAGGCAGTGCTATCCC	ACCATCCCCAGAGTCCAGAAC	TATGCTTCTGGCCGTACCACAGGCA
MYL2	Myosin, light chain 2 (ventricular regulatory light chain 2)	CGGGAGGCAGTGCTGG	GCCCCCGGCTCTCTTCT	TCCTTTCCACCATGGCACCTAAGAAAGC
MYL3	Myosin, light chain 3 (ventricular essential light chain 1)	TGTCATGGGTGCTGAGCTTC	CCACTTCGTCTTCTGTCAGCC	CACGTGCTGGCCACGCTGG
MYL4	Myosin, light chain 4 (atrial essential light chain 1)	ACATGGGTGCTGAGCTTCG	GCTCCACTTCAGCCTCAGTCA	ACGTCCTTGCCACCCTGGGAGAG
MYL7	Myosin, light chain 7, regulatory (MYLC2a, regulatory atrial light chain 2)	TCTTCCTCACGCTCTTTGGG	GGAAGGCACTCAGGATGGC	AAGCTCAATGGGACAGACCCCGAGG
TNNT2	Troponin T type 2 (cardiac)	TCTCCGAAACAGGATCAACGA	GCCCGGTGACTTTAGCCTTC	AACCAGAAAGTCTCCAAGACCCGCG
TNNI3	Troponin I type 3 (cardiac)	CCAACTACCGCGCTTATGC	GCAATTTTCTCGAGGCGGA	ACGGAGCCGCACGCCAAGA
TNNC1	Troponin C type 1 (slow)	GCTGCAGGAGATGATCGA	CATCAAAGTCCACCGTGC	AGGTGGACGAGGACGGCAGC
DMD	Dystrophin	GGACACAAGCACAGGGTTAGA	GGCTTTCCAGGGGTATTTCTT	AGGTGATGGAGCAACTCAACAACTCCT
NPPA	Natriuretic peptide A	AATCCCATGTACAATGCCGTG	TCTTCCAAATGGTCCAGCAAA	CCAACGCAGACCTGATGGATTTCAAGA
NPPB	Natriuretic peptide B	ATGGTGCAAGGGTCTGGCT	TCTTAATGCCGCCTCAGCA	CTTTGGGAGGAAGATGGACCGGATCA
TR-α1	Thyroid hormone receptor, α, splice variant 1	CAGGCTGTGCTGCTAATGTCAA	TTTCATGTGGAGGAAGCGGCT	ACTGACCTCCGCATGAT
TR-α2	Thyroid hormone receptor, α, splice variant 2	CCCAAGCTGCTGATGAAG	CGCTGCCCCCTTGTA	AAGGAGAGAGAAGTGCAGAGTTCGATTCT
ERF	Ets2 repressor factor	TCTCTACAAGTGCCTGCTATCCCTC	TGGGGATGAAGGGGTTGGA	CCACTCCCCACCCCAGCACCG
CSRP3	cysteine and glycine-rich protein 3 (cardiac LIM protein)	GAGAAGGTTATGGGAGGTGGCAA	GTTCCCCATCTTTGTCAGTGACA	ACAAGACCTGTTTCCGCTGTGCCATC
TNF	Tumor necrosis factor	ATCGGCCCGACTATCTCGA	TCCTCACAGGGCAATGATCC	TTTGCCGAGTCTGGGCAGGTCTACTTT
IL1B	Interleukin 1, β	AATCTGTACCTGTCCTGCGTG	TGGGTAATTTTTGGGATCTACACT	TGAAAGATGATAAGCCCACTCTACAGCTGG
CTF1	Cardiotrophin 1	CACCAAATACGCTGAGCAG	CAGCCCGAAGGGGTC	TGCTCCAGGAATATGTGCAGCTCC
IL6	IL6 interleukin 6 (interferon, beta 2)	GCTGTGCAGATGAGTACAAAA	TTATTGCATCTAGATTCTTTGC	TCCTGATCCAGTTCCTGCAGAA
IL6ST	Interleukin 6 signal transducer (gp130)	GCCCTGAATCCATAAAGGCA	CCCTACTTTTTTTGTCCGAACAGT	CCTTAAACAAGCTCCACCTTCCAAAGGACC
NOS2	Nitric oxide synthase 2, inducible	CCTTTACTTGACCTCCTAACAAGTAGC	AGGGAGGCCCCAGTTTGA	CCCTGGATTGATCGGAGCCTCCTC
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	*Life Technologies (Carlsbad, CA) TaqMan Gene Expression Assay : Hs99999905_m1		
RN18S1	RNA, 18S ribosomal 1	*Life Technologies (Carlsbad, CA) TaqMan Gene Expression Assay : Hs99999901_s1		