

## SUPPLEMENTARY MATERIAL

for

“Global Expression Profiling Identifies a Novel Biosignature for Protein Aggregation

R120GCryAB Cardiomyopathy in Mice” Namakkal S. Rajasekaran, Matthew A. Firpo, Brett A.

Milash, Robert B. Weiss and Ivor J. Benjamin

## METHODS

Microarray Printing: PCR products were prepared for spotting by dissolving the samples in a solution of 50% DMSO using a Lucidea Array Spotter (Amersham BioSciences) onto on Corning UltraGAPs slides. The final concentration of these products averaged 100 ng/μl. The spotter contained a 24 pen print set. Each pen, through capillary action when dipped into the spotting solution, takes up less than 200 nl of sample. Glass microscope slides that have been chemically modified with a uniform coating of gamma-amino-propyl silane were positioned on a slide tray of the microarray spotter and held down by vacuum pressure to prevent movement of the slide during the printing process. The physical contact of the spotting pens with the slide surface results in the deposition of approximately 1 nl droplets of microarray printing solution on the slide. The drying rate of these droplets is critical for effective binding of the DNA to the amine groups on the modified slide surface. This is controlled by maintaining 55% relative humidity in the microarray printing compartment and through the hygroscopic properties of DMSO (which slow the drying process). Following completion of a print session, DNA targets were cross-linked to the slide surface at 250 mJ using a Stratagene Stratalinker.

Labeling of Samples: Incorporation of Cy3 or Cy5 fluorescent cyanine dyes was accomplished during first strand cDNA probe synthesis using messenger RNA as a template. Random nonamers were used to prime first strand cDNA synthesis using Superscript II

(Stratagene) in the presence of either Cy3-dCTP or Cy5-dCTP. The resulting cDNA/RNA hybrid was treated with 0.25 M NaOH to hydrolyze RNA. The first strand cDNA molecule was then purified using a Qiagen QIAquick PCR purification kit to remove unincorporated fluorescent nucleotides and primers. The purified labeled sample was subsequently concentrated through speedvac centrifugation.

Hybridization of Labeled Samples: Labeled cDNA was heat denatured at 95°C for 10 minutes and then combined with Ambion SlideHyb Survey Kit Glass Array Hyb Buffer #1 and incubated in a Corning Microarray Hyb cassette overnight at 48°C. The following day, slides were washed as below:

- a. 2X SSC, 0.5% SDS at 48°C for 15 minutes
- b. 2X SSC, 0.5% SDS at 48°C for 15 minutes
- c. 0.5X SSC, 0.5% SDS at 48°C for 15 minutes
- d. 0.5X SSC, 0.5% SDS at 48°C for 15 minutes

Microarray slides were then air-dried by directing compressed air across the surface of the slide.

Microarray Scanning and Image Processing: Microarray slides are scanned in an Axon GenePix 4000B Scanner at a 10 µm/pixel resolution. The Axon scanner simultaneously scans slides using lasers that are optimized for detection of Cy3 and Cy5, resulting in perfect overlay of the two images. This scanner has a dynamic range of detection of four orders of magnitude. The resulting Cy3 and Cy5 images were qualitatively and quantitatively evaluated using ImaGene software (BioDiscovery). This included calculations to make adjustments for spot diameter, calculate localized background values, define and remove artifactual signal, measure spot signal intensity and determine the standard deviation of pixel values within a spot. The software also used various schemas to identify and flag suspicious spots and allow the researcher the option of removing these spots from further consideration.

## TABLES AND FIGURES

**Table S1:** Results of ANOVA Analysis. The number of sequences with altered expression in hCryAB WT, hR120GCryAB or both hCryAB WT and hR120GCryAB relative to the NTG control are listed. Adjusted P-values were determined by controlling the false discovery rate.

Number of Sequences Identified		Correction for Multiple Comparisons
3 Months	6 Months	
127	151	P < 0.05 (uncorrected)
126	151	P < 0.05 (adjusted)
108	126	P < 0.01 (adjusted)
95	114	P < 0.005 (adjusted)

**Table S2:** Gene lists for cellular pathways with > 1.5-fold altered expression in hR120GCryAB hearts relative to hCryAB WT hearts. Ratio refers to the fold change in hR120GCryAB hearts and Direction indicates if the gene was upregulated or down regulated.

	Accession	Gene Symbol	Gene ID	Ratio	Direction	Gene Name
<b>3 MONTH</b>						
Glutathione metabolism						
	BG065030	Gpx1	14775	1.61	up	glutathione peroxidase 1
	BG073718	Gpx3	14778	2.42	up	glutathione peroxidase 3
	BG073190	Gsta4	14860	1.59	up	glutathione S-transferase, alpha 4
	BG086970	Gstm1	14862	1.70	up	glutathione S-transferase, mu 1
	BG074397	Gstm1	14862	1.79	up	glutathione S-transferase, mu 1
	BG086330	Mgst1	56615	1.58	up	microsomal glutathione S-transferase 1
Antigen processing and presentation						
	BG078496	Ctsl	13039	1.60	up	cathepsin L
	BG077017	H2-Eb1	14969	4.07	up	histocompatibility 2, class II antigen E beta
	BG078795	Hspa5	14828	2.12	down	heat shock 70kD protein 5 (glucose-regulated protein)
	BG064772	Hspca	15519	1.62	up	heat shock protein 90kDa alpha (cytosolic), class A member 1
	BG074109	Hspca	15519	1.76	up	heat shock protein 90kDa alpha (cytosolic), class A member 1
	BG064774	Hspca	15519	1.70	up	heat shock protein 90kDa alpha (cytosolic), class A member 1
	BG088007	Hspcb	15516	1.97	up	heat shock protein 90kDa alpha (cytosolic), class B member 1

	BG079631	Hspcb	15516	1.70	up	heat shock protein 90kDa alpha (cytosolic), class B member 1
	BQ550275	li	16149	1.73	up	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)
	BG073636	Psme1	19186	1.50	down	proteasome (prosome, macropain) 28 subunit, alpha
<b>Complement and coagulation cascades</b>						
	BG074814	C1qa	12259	2.02	up	complement component 1, q subcomponent, alpha polypeptide
	BG087868	C1qb	12260	1.96	up	complement component 1, q subcomponent, beta polypeptide
	AW547306	C1qg	12262	1.70	up	complement component 1, q subcomponent, C chain
	BQ553183	F13a1	74145	1.70	up	coagulation factor XIII, A1 subunit
<b>6 MONTH</b>						
<b>Up-regulated</b>						
<b>Ribosome</b>						
	BG085976	Rpl10	110954	1.73	up	ribosomal protein 10
	BG072592	Rpl3	27367	2.03	up	ribosomal protein L3
	BG079511	Rpl3	27367	1.96	up	ribosomal protein L3
	BG072595	Rpl3	27367	2.64	up	ribosomal protein L3
	BG074107	Rpl3	27367	2.31	up	ribosomal protein L3
	BG072985	Rpl7	19989	1.77	up	ribosomal protein L7
	BG063847	Rpl8	26961	1.74	down	ribosomal protein L8
	BG085624	Rps4x	20102	1.77	up	ribosomal protein S4, X-linked
	BG072598	Rps5	20103	1.51	up	ribosomal protein S5
	BG072600	Rps5	20103	1.58	up	ribosomal protein S5
	AW556153	Rps6	20104	1.53	up	ribosomal protein S6
<b>Glutathione metabolism</b>						

	BG073718	Gpx3	14778	1.58	up	glutathione peroxidase 3
	BG073190	Gsta4	14860	1.68	up	glutathione S-transferase, alpha 4
	BG074397	Gstm1	14862	1.59	up	glutathione S-transferase, mu 1
	Bg086330	Mgst1	56615	1.60	up	microsomal glutathione S-transferase 1
	BG088778	Mgst3	66447	1.59	down	microsomal glutathione S-transferase 3
	BG072517	Gstm7	68312	1.69	down	glutathione S-transferase, mu 7
Antigen processing and presentation						
	BG078496	Ctsl	13039	1.68	up	cathepsin L
	BG084164	Grp58	14827	1.68	up	protein disulfide isomerase associated 3
	BG077017	H2-Eb1	14969	2.05	up	histocompatibility 2, class II antigen E beta
	BG064772	Hspca	15519	2.43	up	heat shock protein 90kDa alpha (cytosolic), class A member 1
	BG074109	Hspca	15519	2.86	up	heat shock protein 90kDa alpha (cytosolic), class A member 1
	BG064774	Hspca	15519	1.77	up	heat shock protein 90kDa alpha (cytosolic), class A member 1
	BG063605	Hspca	15519	2.41	up	heat shock protein 90kDa alpha (cytosolic), class A member 1
	BG088007	Hspcb	15516	1.87	up	heat shock protein 90kDa alpha (cytosolic), class B member 1
	BG067038	Hspcb	15516	1.58	up	heat shock protein 90kDa alpha (cytosolic), class B member 1
	BG079631	Hspcb	15516	1.64	up	heat shock protein

						90kDa alpha (cytosolic), class B member 1
	BQ550275	li	16149	1.59	up	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)
	BG073636	Psme1	19186	1.51	down	proteasome (prosome, macropain) 28 subunit, alpha
	BG066650	Psme1	19186	1.51	down	proteasome (prosome, macropain) 28 subunit, alpha
Cell communication						
	BG063870	Actb	11461	1.83	up	actin, beta, cytoplasmic
	C78835	Actb	11461	1.62	up	actin, beta, cytoplasmic
	BG077677	Actb	11461	2.08	up	actin, beta, cytoplasmic
	BG063722	Actb	11461	1.51	up	actin, beta, cytoplasmic
	BG073735	Col1a2	12843	1.81	up	procollagen, type I, alpha 2
	BG074327	Col3a1	12825	2.09	up	procollagen, type III, alpha 1
	BG086357	Col3a1	12825	2.18	up	procollagen, type III, alpha 1
	BG085576	Col3a1	12825	2.10	up	procollagen, type III, alpha 1
	BG088953	Col4a1	12826	1.54	up	procollagen, type IV, alpha 1
	BQ554492	Des	13346	1.62	up	desmin
	BG077621	Lmna	16905	1.52	up	lamin A
Aminoacyl-tRNA biosynthesis						
	C77246	Yars	107271	1.69	up	tyrosyl-tRNA synthetase
	BG079401	Nars	70223	1.51	up	asparaginyl-tRNA synthetase
	BG079434	Sars1	20226	1.53	up	seryl-aminoacyl-tRNA synthetase

<b>Down-regulated</b>						
Oxidative phosphorylation						
	BG069853	Uqcr	66594	1.52	down	ubiquinol-cytochrome c reductase (6.4kD) subunit
	BG086273	Grim19	67184	1.56	down	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13
	BQ553743	Uqcrcfs1	66694	1.55	down	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
	BG074111	Atp5c1	11949	1.83	down	ATP synthase, H <sup>+</sup> transporting, mitochondrial F1 complex, gamma polypeptide 1
	BG088873	Atp5e	67126	1.55	down	ATP synthase, H <sup>+</sup> transporting, mitochondrial F1 complex, epsilon subunit
	BG063439	Atp5h	71679	1.51	down	ATP synthase, H <sup>+</sup> transporting, mitochondrial F0 complex, subunit d
	BG072826	Atp5i	27425	1.71	down	ATP synthase, H <sup>+</sup> transporting, mitochondrial F0 complex, subunit g
	BG086960	Atp6v1h	108664	1.61	up	ATPase, H <sup>+</sup> transporting, lysosomal V1 subunit H
	BG076562	Ndufb7	66916	1.52	down	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7
	BG064317	Cox7b	66142	1.73	down	cytochrome c oxidase subunit VIIb
	BG077969	Ndufs8	225887	1.66	down	NADH dehydrogenase (ubiquinone) Fe-S protein 8
	BG075903	Ndufc1	66377	1.54	down	NADH dehydrogenase (ubiquinone) 1,



						subcomplex unknown, 1
	BQ554627	Ndufa4	17992	1.69	down	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4
	BG086348	Ndufb10	68342	1.64	down	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10
	BG087636	Ndufb9	66218	1.65	down	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9
	BG066265	Ndufs4	17993	1.65	down	NADH dehydrogenase (ubiquinone) Fe-S protein 4
	BG072514	Np15	104130	1.53	down	
	BG084240	Ndufs7	75406	1.69	down	NADH dehydrogenase (ubiquinone) Fe-S protein 7
	BG086026	Sdha	66945	1.86	down	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)
	BG069853	Uqcrc1	22273	1.58	down	ubiquinol-cytochrome c reductase core protein 1
Fatty acid metabolism						
	BG065314	Acadm	11364	1.70	down	acetyl-Coenzyme A dehydrogenase, medium chain
	BG065033	Acads	11409	1.51	down	acyl-Coenzyme A dehydrogenase, short chain
	BG083405	Acat1	110446	1.52	down	acetyl-CoA acetyltransferase
	BG073167	Hsd17b4	15488	2.01	up	hydroxysteroid (17-beta) dehydrogenase 4
	BG069423	Dci	13177	2.17	down	dodecenoyl-Coenzyme A delta isomerase (3,2 trans-enoyl-Coenzyme A isomerase)

	BG079992	Echs1	93747	2.01	down	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial
	BG074754	Acsl1	14081	1.56	down	acyl-CoA synthetase long-chain family member 1
	BG087380	Hadha	97212	1.63	down	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit
	BG086728	Hadhsc	15107	2.33	down	L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain
Carbon fixation						
	BG067706	Gpt2	108682	1.69	down	glutamic pyruvate transaminase (alanine aminotransferase) 2
	BG082516	Fbp2	14120	1.54	down	fructose bisphosphatase 2
	BG078765	Got2	14719	1.66	down	glutamate oxaloacetate transaminase 2, mitochondrial
	BQ550883	Mdh2	17448	1.57	down	malate dehydrogenase 2, NAD (mitochondrial)
	BG067227	Mdh1	17449	2.00	down	malate dehydrogenase 1, NAD (soluble)
	BG064747	Gpt1	76282	1.85	down	glutamic pyruvic transaminase 1, soluble
	BG075310	Tkt	21881	1.56	up	transketolase
Valine, leucine and isoleucine degradation						
	BG065314	Acadm	11364	1.70	down	acetyl-Coenzyme A dehydrogenase, medium chain
	BG065033	Acads	11409	1.51	down	acyl-Coenzyme A

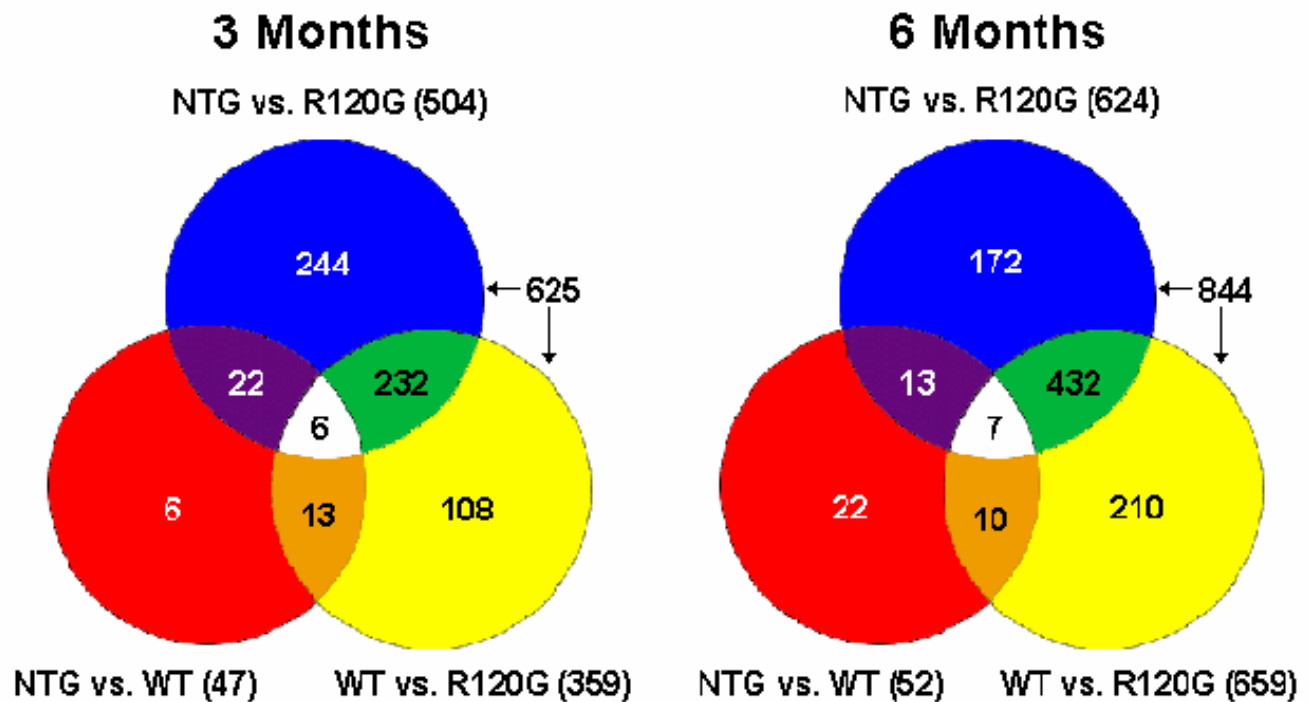
						dehydrogenase, short chain
	BG083405	Acat1	110446	1.52	down	acetyl-CoA acetyltransferase
	BG087153	Bckdha	12039	1.71	down	branched chain ketoacid dehydrogenase E1, alpha polypeptide
	BG073167	Hsd17b4	15488	2.01	up	hydroxysteroid (17-beta) dehydrogenase 4
	BG079992	Echs1	93747	2.01	down	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial
	BG087380	Hadha	97212	1.63	down	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit
	BG086728	Hadhsc	15107	2.33	down	L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain
	BG070984	Ivd	56357	1.79	down	isovaleryl coenzyme A dehydrogenase
Citrate cycle (TCA cycle)						
	BG063733	Cs	12974	1.54	down	citrate synthase
	BQ550883	Mdh2	17448	1.57	down	malate dehydrogenase 2, NAD (mitochondrial)
	BG067227	Mdh1	14779	2.00	down	malate dehydrogenase 1, NAD (soluble)
	BG086026	Sdha	66945	1.86	down	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)
	BG068897	Sucla2	20916	2.17	down	succinate-Coenzyme A ligase, ADP-forming, beta subunit
Pyruvate metabolism						

	BG083405	Acat1	110446	1.52	down	acetyl-CoA acetyltransferase
	BG073920	Ldh2	16832	1.56	down	lactate dehydrogenase B
	BQ550883	Mdh2	17448	1.57	down	malate dehydrogenase 2, NAD (mitochondrial)
	BG067227	Mdh1	17449	2.00	down	malate dehydrogenase 1, NAD (soluble)
	BG068736	Pdha1	18597	1.58	down	pyruvate dehydrogenase E1 alpha 1
Glycolysis / Gluconeogenesis						
	BG075245	Akr1a4	58810	1.54	up	aldo-keto reductase family 1, member A4 (aldehyde reductase)
	BQ554389	Eno3	13808	2.90	down	enolase 3, beta muscle
	BG082516	Fbp2	14120	1.54	down	fructose bisphosphatase 2
	BG073920	Ldh2	16832	1.56	down	lactate dehydrogenase B
	BG068736	Pdha1	18597	1.58	down	pyruvate dehydrogenase E1 alpha 1
	BG088948	Pfkm	18642	1.52	down	phosphofructokinase, muscle
Alanine and aspartate metabolism						
	BG067706	Gpt2	108682	1.69	down	glutamic pyruvate transaminase (alanine aminotransferase) 2
	BG078765	Got2	14719	1.66	down	glutamate oxaloacetate transaminase 2, mitochondrial
	BG064747	Gpt1	76282	1.85	down	glutamic pyruvic transaminase 1, soluble
	BG079401	Nars	70223	1.51	up	asparaginyI-tRNA synthetase

**Table S3:** Comparison of gene expression measures by microarray and Northern blot analyses. Data represents fold-change in hR120GCryAB relative to hCryAB WT controls. Corresponding fold-changes calculated from the Affymetrix microarray validation are shown in parentheses.

	cDNA Microarray Fold-Change (Affymetrix Microarray at 6 months)		Northern Blot Fold-Change	
	3 months	6 months	3 months	6 months
Ankrd1	6.64	8.35 (6.34)	5.74	20.67
Cat	2.26	2.16 (4.20)	4.16	4.49
Gpx3	2.42	N.S. (3.47)	2.51	4.82
Hsp90aa1	N.S.	2.59 (4.20)	3.91	6.02
Eno3	0.49	0.34 (0.41)	0.51	0.59
Mdh1	N.S.	N.S. (N.S.)	0.59	0.59

N.S. = not significant by ANOVA analysis or below 2-fold threshold. However, all such genes were identified as having significant changes at the threshold levels set for the pairwise analysis.



**Figure S1:** Summary of pairwise comparisons at 3 and 6 months. In each Venn diagram, circles represent the individual pairwise comparisons: NTG vs. hCryAB<sup>WT</sup> (red circle), hCryAB<sup>WT</sup> vs. hR120GCryAB (yellow circle), and NTG vs. hR120GCryAB (blue circle). Numbers in parentheses after each comparison represent the total number of sequences with significant change in expression for that comparison. Numbers inside each compartment represents the number of sequences unique for that effect or, if intersecting with another circle, the number of sequences identified for the intersecting set. Note the small number of identified sequences attributable to the hCryAB<sup>WT</sup> compared with the hR120GCryAB transgene.