Supplementary Material

Biomarkers Of Cardiovascular Stress and Fibrosis In Preclinical Hypertrophic Cardiomyopathy

Supplemental Methods

NT-proBNP was measured using the proBNPII immunoassay (Roche, Indianapolis, IN) with a coefficient of variation (CV) of 3.8% at 127 pg/mL and 2.4% at 4180 pg/mL. High-sensitivity TnI was measured using an ultrasensitive immunoassay utilizing a single molecule counting technology (Erenna hsTnI, Singulex, Palo Alto, CA) with CV of 12.5% at 6.8 ng/L and 13.7% at 39.2 ng/L. Soluble ST2 was measured using a sandwich immunoassay (Presage^{TM} ST2, Critical Diagnostics, San Diego, CA) with CV of 14.0% at 24.8 ng/mL and 10.6% at 76.6 ng/mL. Galectin-3 concentrations were measured using an ELISA assay (BG Medicine, Waltham, MA, USA) with CV of 6.4% at 20.4 µg/L and 6.6% at 77.1 µg/L. PICP was measured using ELISA (Quidel Corporation, San Diego, CA) with a detectable threshold of 0.20 µg/L and inter-assay CV 7.0% and 5.0% at low and high concentrations, respectively, and an intra-assay CV of 6.4%. CITP was measured using ELISA (Orion Diagnostica, Espoo, Finland) with a detectable threshold of 0.30 µg/L, and inter-assay CV's of 8.2 and 10.1% at low and high concentrations, respectively, and intra-assay CV <10%. Bone alkaline phosphatase (BAP) was measured using an enzymatic assay (BioVision, Mountain View, CA) with a detectable threshold of 0.25 U/mL, inter-assay CV's of 9.1 and 7.3% at low vs. high concentrations, and intra-assay CV of 8.6%.

Supplemental Table 1. HCM♥Net Participating Sites and Enrollment

Site	**Site PI**	**N included***
Brigham and Women's Hospital	Carolyn Ho, MD	61
Boston Children's Hospital	Steve Colan, MD	43
Cleveland Clinic Foundation	Harry Lever, MD	2
Cincinnati Children's Hospital Medical Center	Jeff Towbin, MD	9
University of Michigan	Sharlene Day, MD Mark Russell, MD	30
University of Chicago	Elizabeth McNally, MD	0
St. Luke's-Roosevelt Hospital Center	Mark Sherrid, MD Bette Kim, MD	10
Johns Hopkins University	Anne Murphy, MD	2
Washington University School of Medicine	Charles Canter, MD	10
University of Colorado	Matthew Taylor, MD Luisa Mestroni, MD	0
Stanford University	Euan Ashley, MRCP DPhil	0

*Some sites enrolled participants for the main study sample, however were excluded due to missing biomarker measures or other exclusion criteria specific to this analysis

Supplemental Table 2. Biomarker comparisons among those with *MYH7* versus *MYBPC3* mutations

	MYH7 **(n=46)**	***MYBPC3*** **(n=65)**	**P***
Baseline biomarkers, median (25^{th}-75^{th} percentile)			
NT-proBNP, pg/ml	71 (45, 201)	68 (31, 181)	0.71
High-sensitivity troponin, ng/ml	4.0 (1.5, 6.8)	4.2 (1.8, 10.1)	0.46
Soluble ST2, ng/ml	25.1 (17.5, 30.6)	28.5 (21.8, 37.1)	0.06
Galectin-3, pg/ml	11.2 (9.5, 13.3)	11.4 (9.8, 13.1)	0.66
PICP, ug/L	105.4 (76.9, 146.2)	90.2 (74.6, 132.6)	0.42
CITP, ug/L	8.1 (3.6, 12.7)	4.6 (3.1, 10.4)	0.73
PICP/CITP ratio	15.2 (10.3, 24.2)	18.7 (11.8, 31.2)	0.80
BAP, U/L	1.7 (1.2, 2.9)	1.3 (1.1, 1.9)	0.11
PICP/BAP ratio	57.9 (33.7, 111.3)	61.5 (46.1, 109.6)	0.20
Immediate or 4-hour post-exercise biomarkers, median (25^{th}-75^{th} percentile)			
NT-proBNP, pg/ml	88 (54, 221)	91 (42, 233)	0.78
Change in NT-proBNP, pg/ml	14 (6, 28)	15 (7, 62)	0.93
4-hour hsTnI, ng/ml	5.4 (1.7, 8.8)	6.6 (2.4, 14.2)	0.57
Change in hsTnI, ng/ml	0.3 (-0.3, 1.7)	1.3 (-0.1, 5.3)	0.67
4-hour soluble ST2, ng/ml	31.7 (23.9, 38.8)	37.3 (27.7, 45.0)	0.08
Change in sST2, ng/ml	4.9 (1.2, 9.9)	7.8 (1.5, 11.1)	0.29

* Adjusted for age, sex, and familial correlation

Supplemental Table 3. Biomarker comparisons among those with thick versus thin filament mutations

	Thick Filament (n=116)	Thin Filament (n=10)	P*
Baseline biomarkers, median (25^{th}-75^{th} percentile)			
NT-proBNP, pg/ml	72 (41.5, 183.5)	251.5 (30, 821)	0.39
High-sensitivity troponin, ng/ml	4.3 (1.6, 8.9)	7.0 (1.9, 9.5)	0.28
Soluble ST2, ng/ml	26.5 (20.2, 34.7)	43.8 (33.1, 48.6)	0.05
Galectin-3, pg/ml	11.4 (9.7, 13.3)	10.8 (10.1, 13.2)	0.82
PICP, ug/L	100.2 (75.3, 144.3)	91.4 (79.9, 126.3)	0.49
CITP, ug/L	5.4 (3.1, 11.5)	3.6 (3.2, 4.4)	0.10
PICP/CITP ratio	17.3 (11.3, 30.0)	24.6 (17.3, 34.7)	0.93
BAP, U/L	1.4 (1.1, 2.4)	2.1 (1.0, 3.0)	0.86
PICP/BAP ratio	61.2 (39.3, 111.9)	57.1 (33.2, 79.9)	0.80
Immediate or 4-hour post-exercise biomarkers, median (25^{th}-75^{th} percentile)			
NT-proBNP, pg/ml	93 (46, 224)	305.5 (47, 1184)	0.37
Change in NT-proBNP, pg/ml	15 (6, 51)	59 (17, 133)	0.40
4-hour hsTnI, ng/ml	6.4 (2.2, 12.5)	9.4 (5.5, 16.7)	0.17
Change in hsTnI, ng/ml	0.8 (-0.3, 3.3)	1.5 (0.6, 2.4)	0.17
4-hour soluble ST2, ng/ml	35.1 (25.5, 42.8)	41.5 (36.0, 51.2)	0.32
Change in sST2, ng/ml	7.8 (1.4, 10.9)	2.1 (-0.7, 4.6)	0.02

* Adjusted for age, sex, and familial correlation