Supplemental Material

Prediction of Sarcomere Mutations in Subclinical Hypertrophic Cardiomyopathy

Captur et al: Subclinical Phenotype in HCM

Gabriella Captur, MD, MRCP, MSc[1,2,3]; Luis R. Lopes, MD[1,2]; Timothy J. Mohun, PhD[4]; Vimal Patel, MD, MRCP[1,2]; Chunming Li, PhD[5]; Paul Bassett, MSc[6]; Gherardo Finocchiaro, MD[3]; Vanessa M. Ferreira, MD, DPhil, FRCPC[7]; Maite Tome Esteban, MD[2,3]; Vivek Muthurangu, MD, MRCP[1,8]; Mark V. Sherrid, MD[9]; Sharlene M. Day, MD[10]; Charles E. Canter, MD[11]; William J. McKenna, MD, FACC, FRCP[1,2,3]; Christine E. Seidman, MD[12]; David A. Bluemke, MD, PhD[13]; Perry M. Elliott, MD, FRCP, FESC, FACC[1,2]; Carolyn Y. Ho, MD[14]; James C. Moon, MB, BCh, MRCP, MD.[1,2,3]

[1]Institute of Cardiovascular Science and [6]Biostatistics Joint Research Office, University College London, London, United Kingdom

[2]The Inherited Cardiovascular Diseases Unit and [3]Cardiac Imaging Department, The Barts Heart Centre, London, United Kingdom

[4]Department of Developmental Biology, MRC National Institutes for Medical Research, Mill Hill, United Kingdom

[5]Department of Radiology, University of Pennsylvania, Philadelphia, PA

[7]University of Oxford Center for Clinical Magnetic Resonance Research (OCMR), Division of Cardiovascular Medicine, Radcliffe Department of Medicine John Radcliffe Hospital, Oxford, United Kingdom

[8]UCL Center for Cardiovascular Imaging and Great Ormond Street Hospital for Children, London, United Kingdom

[9]Mount Sinai Roosevelt Hospital, Icahn School of Medicine at Mount Sinai, New York, NY

[10]Department of Internal Medicine, University of Michigan Medical Center, Michigan, Ann Arbor, MI

[11]Washington University School of Medicine, St Louis, MO

[12]Department of Genetics, Harvard Medical School, Boston, MA

[13]Radiology and Imaging Sciences, National Institutes of Health/Clinical Center, Bethesda, MD

[14]Cardiovascular Division, Brigham and Women's Hospital, Boston, MA and representing the HCMNet Investigators

Expanded methods:

Note S1. The list of HCMNet investigators.

(n = X) Refers to the combined number of G+LVH− cases and controls recruited from within each of the respective HCMNet centers (total n = 49).

1. Stanford University (n = 1). Stanford, California, United States, 94305

Contact: Euan Ashley, MD, PhD 650-498-4900 euan@stanford.edu

Principal Investigator: Euan Ashley, MD, PhD

2. University of Chicago (n = 0). Chicago, Illinois, United States, 60637

Contact: Elizabeth McNally, MD 773-702-2672 emcnally@uchicago.edu

Principal Investigator: Elizabeth McNally, MD

3. Johns Hopkins University (n = 0). Baltimore, Maryland, United States, 21287

Contact: Anne Murphy, MD 410-955-5987 murphy@jhmi.edu

Principal Investigator: Anne Murphy, MD

Sub-Investigator: Ted Abraham, MD

4. Brigham & Women's Hospital (n = 21). Boston, Massachusetts, United States, 02115

Contact: Carolyn Ho, MD 617-732-5685 cho@partners.org

Principal Investigator: Carolyn Ho, MD

Sub-Investigator: Mark A. Fifer, MD

5. Children's Hospital Boston (n = 1). Boston, Massachusetts, United States, 02115

Contact: Steven Colan, MD 617-355-7893 colan@alum.mit.edu

Principal Investigator: Steven Colan, MD

Sub-Investigator: Renee Margosian, MD

6. University of Michigan (n = 12). Ann Arbor, Michigan, United States, 48109

Contact: Sharlene Day, MD 734-615-7917 sday@umich.edu

Principal Investigator: Sharlene Day, MD

Sub-Investigator: Mark Russell, MD

7. Washington University School Medicine (n = 6). St. Louis, Missouri, United States, 63110

Contact: Charles Canter, MD 314-454-6095 canter@kids.wustl.edu

Principal Investigator: Charles Canter, MD

Sub-Investigator: Keith Mankovitz, MD

8. Cincinnati Children's Hospital Medical Center (n = 2). Cincinnati, Ohio, United States, 45229

Contact: Jeffrey Towbin, MD 513-636-3049 jeffrey.towbin@cchmc.org

Principal Investigator: Jeffrey Towbin, MD

Sub-Investigator: John Lynn Jeffries, MD

9. Cleveland Clinic Foundation (n = 0). Cleveland, Ohio, United States, 44195

Contact: Harry Lever, MD 216-444-6970 leverh@ccf.org

Principal Investigator: Harry Lever, MD

Sub-Investigator: Kenneth Zahka, MD

10. St. Luke's Roosevelt Hospital Center (n = 6). New York, United States, 10019

Contact: Mark Sherrid, MD 212-492-5550 msherrid@chpnet.org

Principal Investigator: Mark Sherrid, MD

11. University of Colorado (n = 0). Aurora, Colorado, United States, 80045

Contact: Matthew Taylor, MD, PhD 303-724-1400 matthew.taylor@ucdenver.edu

Principal Investigator: Matthew Taylor, MD, PhD

Sub-Investigator: Luisa Mestroni, MD

Note S2. Deriving the variable LVESVi_R for entry into the predictive model

The gender and age specific model variable LVESVi_R is computed as a ratio of the measured body surface area (BSA)-indexed LVESV ($_{measured}$LVESV*i*, ml/m^2) divided by the expected BSA-indexed LVESV ($_{expected}$LVESV*i*, ml/m^2). Values for expected BSA-indexed LVESV have been previously published,[1] and are summarized here for ease of reference.

Age Range	**Expected LVESV*i*** (ml/m^2)	
(years)	**Males**	**Females**
10 – 19*	32	28
20 – 29	30	28
30 – 39	29	27
40 – 49	27	25
50 – 59	26	24
60 – 69	25	22
70 – 79	24	21

Equation: $LVESVi_R = (_{measured}LVESVi / _{expected}LVESVi)$

* Expected LVESV*i* values for children[2] (ages 10-19) are derived from the normative equations published by the group (expected value = a*BSA^b, where *a* represents a gender factor, and *b* a regression model derivative).

Note S3. Deriving the fractal dimension, dependency on slice-thickness and magnetic field strength

Fractal analysis of cine cardiovascular magnetic resonance (CMR) data sets was performed in MATLAB® (The MathWorks Inc., Natick, MA, USA) using the method previously described by our group.[3,4] Briefly, the end-diastolic LV short-axis cine frame is scaled according to DICOM pixel spacing metadata; the region of interest is defined; endocardial border extraction proceeds by a region-based level-set segmentation[5] and implementation of a standard box-counting algorithm derives the maximal apical fractal dimension (FD). To investigate the impact of varying slice thickness on FD we compared the FD of 43 cine slices each acquired at 6 mm, 7 mm and 8 mm thicknesses respectively (scans performed at University College London). To investigate the impact of varying magnetic field strength on FD, we compared the FD of 134 cine slices each acquired at 1.5 and 3-Tesla (mixed healthy and diseased cohorts; scans performed at National Institutes of Health and University of Oxford Center for CMR). Analysis of variance with repeated measures determined that mean FD did not differ significantly between the 3 slice thicknesses ($F(2, 84) = 2.259$, $P = 0.1$). Agreement between raw FD values was good irrespective of magnetic field strength (intraclass correlation coefficients, 0.92; 95% confidence intervals, 0.90 – 0.94).

Note S4. Repeated analyses for unrelated family members

To ensure that differences in CMR parameters between G+LVH− and controls were not driven by the degree of relatedness, the difference testing and univariable logistic regression analyses were repeated after randomly retaining only one subject per family. Results in this unrelated case-control population (n = 62 pairs, Supplemental Tables 4 and 5) confirmed these relationships independent of the degree of relatedness.

Table S1. Genetic and electrocardiographic characteristics across all G+LVH− (n = 73).

No.	Gene Family†	Amino acid/ Coding Sequence Change	Romhilt-Estes Score
1	*MYBPC3*	NM_000256:p.R495G	0
2	*TNNT2*	NM_001001432:p.R278C	3
3	*MYBPC3*	NM_000256:p.E542Q	0
4	*MYBPC3 & TNNI3*	NM_000256:p.E542Q and NM_000363:p.R162Q	0
5	*MYBPC3*	NM_000256:p.G969X	3
6	*MYBPC3*	NM_000256:p.Q791fs	0
7	*ACTC1*	NM_005159:p.R314C	0
8	*MYH7*	NM_000257:p.G741R	0
9	*TNNT2*	NM_001001432:p.R278C	0
10	*MYH7*	NM_000257:p.A797T	0
11	*TNNT2*	NM_001001432:p.R278C	0
12	*MYH7*	NM_000257:p.R787H	0
13	*MYH7*	NM_000257:p.R663H	0
14	*MYBPC3*	NM_000256:c.IVS14-13G>A	0
15	*ACTC1*	NM_005159:p.R314C	0
16	*MYBPC3*	NM_000256:p.R495G	0
17	*MYH7*	NM_000257:p.V606L	0
18	*TNNI3*	NM_000363:p.A157V	0
19	*TNNT2*	NM_001001432:p.R278C	3
20	*MYBPC3*	NM_000256:p.K1055X	0
21	*MYBPC3*	NM_000256:p.K1055X	3
22	*TNNI3*	NM_000363:p.R141G	0
23	*MYBPC3*	NM_000256:p.Q791fs	0
24	*TNNI3*	NM_000363:p.R145W	0
25	*TNNI3*	NM_000363:p.R145W	0
26	*TNNI3*	NM_000363:p.R145W	0
27	*TNNI3*	NM_000363:p.A157V	0
28	*MYBPC3*	NM_000256:c.IVS11-2A>G	0
29	*MYH7*	NM_000257:p.E927K	0
30	*MYBPC3*	NM_000256:p.Q791fs	0
31	*MYH7*	NM_000257:p.A355T	0
32	*MYBPC3*	NM_000256:c.IVS9-1G>C	0
33	*MYBPC3*	NM_000256:c.IVS11-2A>G	0
34	*MYBPC3*	NM_000256:p.P955fs and c.IVS1-154T>C	0
35	*MYBPC3*	NM_000256:p.E542Q	2
36	*TNNT2*	NM_001001432:p.R278C	0
37	*MYBPC3*	NM_000256:p.Q791fs	0
38	*MYBPC3*	NM_000256:p.R502G	0
39	*TNNI3*	NM_000363:p.R145W	3
40	*TNNT2*	NM_001001432:p.R278C	2

41	*MYBPC3*	NM_000256:p.E258K	1
42	*MYH7*	NM_000257:p.R652G	0
43	*MYBPC3*	NM_000256:p.W683X	1
44	*MYH7*	NM_000257.p.V606M	1
45	*MYBPC3*	NM_000256:p.F412X	0
46	*MYH7*	NM_000257:p.R663C	1
47	*MYH7*	NM_000257:p.R663C	1
48	*MYBPC3*	NM_000256:p.R495G	0
49	*MYH7*	NM_000257:p.H581R	1
50	*MYBPC3*	NM_000256:p.F631fs	0
51	*MYH7*	NM_000257:p.R858S	3
52	*MYL3*	NM_000258:p.A57G	3
53	*MYH7*	NM_000257:p.G741R	1
54	*MYH7*	NM_000257:p.S291F	1
55	*TNNT2*	NM_001001432:p.R278C	1
56	*TNNI3*	NM_000363:p.T143N	1
57	*MYH7*	NM_000257:p.V338M	1
58	*MYH7*	NM_000257:p.R858G	1
59	*MYBPC3*	NM_000256:p.S858N	0
60	*MYBPC3*	NM_000256:p.S858N	0
61	*MYBPC3*	NM_000256:p.E258K	0
62	*MYBPC3*	NM_000256:p.E258K	0
63	*MYH7*	NM_000257:p.R663C	3
64	*MYH7*	NM_000257:p.R249Q	0
65	*MYBPC3*	NM_000256:p.W683X	0
66	*MYH7*	NM_000257:p.E497D	0
67	*MYBPC3*	NM_000256:p.E60AfsX49	0
68	*MYBPC3*	NM_000256:p.F631fs	0
69	*MYH7*	NM_000257:p.I323N	1
70	*ACTC*	NM_005159:p.E101K	1
71	*MYBPC3*	NM_000256:p.R502Q	0
72	*MYH7*	NM_000257:p.R858S	1
73	*MYH7*	NM_000257:p.A797T	0

† Though screening for sarcomere gene mutations was only largely performed on the G+LVH− and the 2 genotype-negative family members, the prevalence of such mutations in the rest of the control population is expected to be low.[6]

ACTC1 = actin, alpha cardiac muscle 1; G+LVH− = genotype-positive, left ventricular hypertrophy-negative; *MYBPC3* = myosin-binding protein C, cardiac type; *MYH7* = myosin heavy chain, cardiac muscle beta isoform; *MYL3* = myosin light polypeptide 3*; TNNT2* = troponin T, cardiac muscle; *TNNI3* = troponin I, cardiac muscle.

Table S2. Typical cine steady-state free precession imaging parameters for the different magnet manufacturers used across the 12 participating institutions.

Manufacturer	**Siemens**		**General Electric**	**Philips**
	Avanto	**Tim Trio**	**Signa Excite**	**Achieva**
Slice thickness (mm)	7/8	8	6	8
Inter-slice gap (mm)	3/2	0	1	0
Flip angle (°)	80	37	70	60
TR (ms)	3.13	3.68	3.72	2.63
TE (ms)	1.33	1.17	1.62	1.31
FOV read (mm)	380	360	360	360
% Phase FOV	80	90	80	100

FOV = field of view; TE = echo time; TR = repetition time.

Table S3. Tests for difference repeated for the 4 predictive parameters but considering only unrelated carriers and their matched controls (n = 62 pairs).

Variable	**G+LVH−**	**Control**	**p-Value for Difference Test**
≥ 1 Crypt Present n (%)	21 (34)	1 (2)	< 0.0001*
AMVL (mm)	23.2 ± 3.0	21.0 ± 2.9	< 0.001
$FD_{MaxApical}$	1.257 ± 0.07	1.197 ± 0.05	< 0.0001
LVESVi_R	0.83 ± 0.2	0.94 ± 0.2	0.006

***** Using Fisher's exact test for binary variable '≥ 1 Crypt Present' and paired *t*-Test for remaining variables.

Values expressed as mean ± standard deviation except where otherwise stated.

Abbreviations as in Tables S1 and S3.

Table S4. Univariable analysis repeated in the unrelated cohort (n = 62) for the association of the 4 key parameters with genetic status.

Variable	**Crude OR (95% CI)**	**p-Value (Wald χ^2)**
AMVL (mm)	1.27 (1.10, 1.46)	0.001
$FD_{MaxApical}$*	5.33 (2.21, 12.87)	< 0.001
LVESVi_R	0.09 (0.15, 0.58)	0.011
≥ 2 Crypts Present (Y/N) †	27.66 (3.49, 3614)	< 0.001

* Coefficients are expressed for each 0.1 unit change in $FD_{MaxApical}$.

† Using Firth's bias-controlled logistic regression on account of complete separation.

Abbreviations as in Table S3.

Table S5. Multivariable conditional logistic regression model that includes the predictor '≥ 1 Crypt Present'.

Variable	**β Coefficient**	**Adjusted OR (95% CI)**	**p-Value (Wald χ^2)**
≥ 1 Crypt Present	2.45	11.54 (1.63, 81.78)	0.014
AMVL	2.34	10.95 (2.66, 45.12)	< 0.001
$FD_{MaxApical}$	1.92	6.80 (1.51, 30.6)	0.013
LVESVi_R†	1.48	4.39 (1.31, 14.68)	0.02

† LVESVi_R and other model covariates entered as categorical (binary) predictors using Youden-derived cut-offs. AMVL = anterior mitral valve leaflet; β = beta; χ^2= Chi-squared; CI = confidence interval; $FD_{MaxApical}$ = maximal apical fractal dimension; LVESVi_R = left ventricular end-systolic volume adjusted for body size, age and gender; OR = odds ratio.

Supplemental References:

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6. Maron BJ. Hypertrophic cardiomyopathy: a systematic review. *JAMA*. 2002; 287:1308–20.