Supplementary Online Content

Maurizi N, Passatino S, Spaziana G, et al. Long-term outcomes of pediatric-onset hypertrophic cardiomyopathy and age-specific risk factors for lethal arrhythmic events. *JAMA Cardiol*. Published online April 18, 2018. doi:10.1001/jamacardio.2018.0789.

This supplementary material has been provided by the authors to give readers additional information about their work.

Methods

Demographics

Ninety-eight of the 100 patients included in the present cohort were Caucasian white individuals. The remaining two patients were of South America origin but born in Italy.

Baseline Assessment and Follow-Up

Initial clinical evaluation was defined as the time when the diagnosis of HCM was first confirmed and included two-dimensional echocardiography, 12-lead ECG and 24- to 48-h ambulatory (Holter) ECG. Two-dimensional, Doppler, and M-mode echocardiography was performed at rest using standard methods. Left ventricular outflow tract obstruction was defined as a peak resting gradient >30 mmHg. Treadmill exercise was performed at baseline in children aged 6 or more. Cardiac magnetic resonance imaging (CMR) was not routinely performed in children, unless required e.g. for surgical planning or to exclude anomalous coronary origin or bridging. Functional status was carefully assessed in all patients at baseline and at follow-up visits by one of the senior cardiologists. Presence of symptoms was defined by any degree of limitation in physical activities, interfering with ordinary activities and resulting in tachycardia, fatigue or dyspnea (1-3). For patients <9 years of age, modified Ross criteria were used (1). A Ross score >2 points defined a symptomatic status equivalent to NYHA Functional Class II or more (2,3). In patients >9 years, standard functional stratification according to NYHA class was used. Genetic counseling and mutational analysis were routinely offered to all patients and their families since 2000 (Online Supplement) (4).

Exclusion of HCM Mimics

Great care was taken to exclude children suspected to have an increase in LV wall thickness secondary to a metabolic/infiltrative disease, malformation syndromes, or neuromuscular disorders. **Unless the children had a pathogenic/likely pathogenic mutation in a sarcomere gene, or a family history of HCM, they were referred to our metabolic specialist for clinical and laboratory assessment. As a result, classic HCM was excluded in 33 children and adolescents over the years, including 2 due to incongruous clinical traits and pattern of inheritance in the family, 21 because of positive genetic screening for HCM**

phenocopies and 10 due to in mitochondrial DNA variants. In addition, hearts of children who underwent heart transplantation were systematically examined to exclude infiltrative or storage diseases (5).

Follow-Up Strategy

Follow-up and management strategies were generally consistent throughout the period of follow-up. Patients were generally followed up in a standard fashion at 1-year intervals with clinical examination, two-dimensional echocardiography, 12-lead ECG, 24- to 48-h ambulatory (Holter) ECG and treadmill exercise test if clinically indicated.

Genetic Testing

Genetic counseling and mutational analysis were routinely offered to all patients and their families since 2000. Following informed consent, patients were screened by Sanger for mutations in the protein-coding exons and splice sites of 8 myofilament genes, including myosin binding protein C (MYBPC3), beta-myosin heavy chain (MYH7), the regulatory and essential light chains (MYL2 and MYL3), troponin-T (TNNT2), troponin-I (TNNI3), alpha-tropomyosin (TPM1), and alpha-actin (ACTC). Direct deoxyribonucleic acid (DNA) sequencing was performed using ABI-Prism 3730 (Applied Biosystems, Foster City, California). Since 2013, target sequencing in disease specific panels were performed using next generation sequencing techniques (8 sarcomeric genes and *LAMP2, GLA, PRKGAG2, TTR*). Variants were classified according to 2015 ACMG Guidelines (4), specifically

Statistical Methods

Statistical analysis was performed using SPSS version 21 (IBM Corporation, Armonk, New York) and R version 3.3.1 (R Foundation, Vienna, Austria). Data are expressed as percentage, mean and SD, or median with interquartile range (IQR) for skewed distributions. Patients diagnosed with HCM at older ages, by virtue of having survived to the time of diagnosis, could not have had an event between birth and the time of diagnosis. This phenomenon, called delayed entry or left-truncation, is common in studies where the time variable of interest is the age of an individual (30). To avoid this bias, we removed patients from the risk set between birth and diagnosis of HCM, and considered only the time

during which patients were followed prospectively (31). To highlight the behavior of HCM in different age groups, we reported incidence rates for cardiovascular mortality in each decade.

Incidence rates were computed by dividing the number of patients experiencing a cardiovascular death by the total number of person-years. Also, to adjust for delayed entries, the time from birth to diagnosis was not considered in the person-years calculation. During follow-up, we recorded LAE occurrence and the cumulative probability of a first LAE during follow-up was determined with the life-table method of Kaplan-Meier, and results were compared with the log-rank test. Patients were censored at last visit or at the occurrence of death for non-arrhythmic causes. Prognostic factors for LAE at follow-up were assessed by univariable analysis and characteristics significantly ($p < 0.05$) or nearly significantly ($p < 0.10$) associated with LAE in the univariable analysis were first entered as candidate variables in a multivariate Cox proportional hazards regression analysis. The final multivariable model was selected using a backward-elimination algorithm (retention threshold $p < 0.05$).

References:

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Online Tables and Figures:
eTable 1. Genetic Test results in Pediatric-Onset Hypertrophic Cardiomyopathy.

A pathogenic/likely pathogenic mutation was found in 56 of 70 patients (80%). Mutations were most frequent in genes encoding for thick filament structures, particularly, MYBPC3, MYH7 and MYL2. Thin filament mutations were present in 9/56 patients and one patient had a mutation on JPH2 gene. A complex genotype (MYBPC3-P371R and MYH7-R869K) was present in two sisters (8 and 6 years old respectively).

PATIENTS	*GENETIC VARIANTS*	*VARIANTS CLASSIFICATION*
PT001	Not genotyped	
PT002	Not genotyped	
PT003	MYBPC3 c.1484G>A p.(Arg495Gln)	Pathogenic/likely pathogenic
PT004	MYH7 c.1304T>G p.(Met435Arg)	Pathogenic/likely pathogenic
PT005	MYH7 c.1304T>G p.(Met435Arg)	Pathogenic/likely pathogenic
PT006	MYH7 c. 2302G>A p.(Gly768Arg)	Pathogenic/likely pathogenic
PT007	Not genotyped	
PT008	MYL2 c.173G>A p.(Arg58Gln)	Pathogenic/likely pathogenic
PT009	MYL2 c.173G>A p.(Arg58Gln)	Pathogenic/likely pathogenic
PT010	TNNI3 c.592C>G p.(Leu198Val)	Pathogenic/likely pathogenic
PT011	MYBPC3 c.1505G>A p.(Arg502Gln)	Pathogenic/likely pathogenic
PT012	MYBPC3 c.772G>A (p.Glu258Lys)	Pathogenic/likely pathogenic
PT013	MYBPC3 c.ivs7+1g>a p.(?), MYBPC3 c.1828C>G (p.Asp610His)	Pathogenic/likely pathogenic
PT014	Not genotyped	
PT015	MYBPC3 c.3808G>A p.(Val1270Met)	Pathogenic/likely pathogenic
PT016	MYH7 c.2606G>A p.(Arg869His)*	Pathogenic/likely pathogenic
PT017	Not genotyped	
PT018	Not genotyped	
PT019	MYBPC3 c.3192dup p.(Lys1065Glnfs*1), MYBPC3 c.1112C>G p.(Pro371Arg)	Pathogenic/likely pathogenic
PT020	MYBPC3 c.3192dup p.(Lys1065Glnfs*1), MYBPC3 c.1112C>G p.(Pro371Arg)	
PT021	MYH7 c.108G>A p.(Arg403Gln)	Pathogenic/likely pathogenic
PT022	MYBPC3 c.2870 C>G p.(Thr957Ser)	Pathogenic/likely pathogenic
PT023	MYBPC3 c.1505G>A p.(Arg502Gln)	Pathogenic/likely pathogenic
PT024	Not genotyped	
PT025	negative test	
PT026	negative test	
PT027	Not genotyped	
PT028	Not genotyped	
PT029	MYBPC3 c.2311G>A p.(Val771Met)	Pathogenic/likely pathogenic
PT030	MYH7 c.2779G>A p.(Glu927Lys)	Pathogenic/likely pathogenic
PT031	MYH7 c.2779G>A p.(Glu927Lys)	Pathogenic/likely pathogenic
PT032	Negative test	
PT033	Not genotyped	
PT034	Not genotyped	
PT035	MYL2 c.58A>C p.(Met20Leu)	Pathogenic/likely pathogenic
PT036	MYL2 c.58A>C p.(Met20Leu)	Pathogenic/likely pathogenic
PT037	TNNI3 c.611G>A (p.Arg204His)	Pathogenic/likely pathogenic

PT038	MYH7 c.108G>A p.(Arg403Gln)	Pathogenic/likely pathogenic
PT039	MYH7 c.108G>A p.(Arg403Gln)	Pathogenic/likely pathogenic
PT040	Not genotyped	
PT041	Not genotyped	
PT042	MYBPC3 c.2905+1G>A (p?)	VUS
PT043	Not genotyped	
PT044	Not genotyped	
PT045	MYH7c.1816G>A p.(Val606Met)	Pathogenic/likely pathogenic
PT046	Not genotyped	
PT047	MYH7 c.1433T>A p.(Ile478Asn)	Pathogenic/likely pathogenic
PT048	MYH7 c.2156G>A (p.Arg179Gln),SCN5A c.436G>A p.(Val146Met)	Pathogenic/likely pathogenic
PT049	MYH7c.1208G>A (p.Arg403Gln), MYH7 c.2890G>C p.(Val964Leu)	VUS
PT050	TNNI3 c.575G>T p.(Arg192Leu)	Pathogenic/likely pathogenic
PT051	Negative test	
PT052	MYBPC3 c.2905+1G>A (p?)	Pathogenic/likely pathogenic
PT053	MYH7 c.2606G>A p.(Arg869His)	Pathogenic/likely pathogenic
PT054	Negative test	
PT055	Negative test [but positive for JPH2 c.505G>A (p.Glu169Lys)]**	
PT056	Negative test	
PT057	MYBPC3 c.1624G>C (p.Glu542Gln)	Pathogenic/likely pathogenic
PT058	TNNT2 c.360T>C p.(Phe120Leu)	Pathogenic/likely pathogenic
PT059	TNNT2 c.275G>A p.(Arg92Gln)	Pathogenic/likely pathogenic
PT060	Not genotyped	
PT061	MYBPC3 c.772G>A (p.Glu258Lys)	Pathogenic/likely pathogenic
PT062	*Negative test*	
PT063	MYH7c.697G>T p.(Ala233Ser)	Pathogenic/likely pathogenic
PT064	TNNI3 c.557G>A p.(Arg186Gln)	Pathogenic/likely pathogenic
PT065	*Negative test*	
PT066	*Negative test*	
PT067	MYBPC3 c.1575T>A p.(Tyr525*)	Pathogenic/likely pathogenic
PT068	Not genotyped	
PT069	Not genotyped	
PT070	MYH7 c.1826A>G (p.Val698Ala)	Pathogenic/likely pathogenic
PT071	MYH7 c.2573G>C p.(Arg858Pro)	VUS
PT072	Not genotyped	
PT073	Not genotyped	
PT074	MYBPC3 c.1505G>A p.(Arg502Gln)	Pathogenic/likely pathogenic
PT075	MYBPC3 c.1505G>A p.(Arg502Gln)	Pathogenic/likely pathogenic
PT076	MYBPC3 c.1505G>A p.(Arg502Gln)	Pathogenic/likely pathogenic
PT077	Negative test	
PT078	MYBPC3 c.1484G>A p(.Arg495Gln), MYL3 c.170C>A p.(Ala57Asp), SCN5A c.4057G>A p.(Val1353Met)	Pathogenic/likely pathogenic
PT079	TNNT2 c.310C>T p.(Arg104Cys)	Pathogenic/likely pathogenic
PT080	Negative test	
PT081	MYL2 c.484G>A p.(Gly162Arg)	Pathogenic/likely pathogenic
PT082	MYL2 c.484G>A p.(Gly162Arg)	Pathogenic/likely pathogenic
PT083	TNNT2 c.280C>T(p.Arg94Cys)	Pathogenic/likely pathogenic
PT084	*Not genotyped*	
PT085	*Not genotyped*	

PT086	TNNT2 c.304C>T p.(Arg102Trp)	Pathogenic/likely pathogenic
PT087	*Not genotyped*	
PT088	*Not genotyped*	
PT089	*Not genotyped*	
PT090	*Not genotyped*	
PT091	*Not genotyped*	
PT092	MYH7 c.2167C>T p.(Arg723Cys) PRKAG2 c.298G>A p.(Gly100Ser)	Pathogenic/likely pathogenic
PT093	MYBPC3 c.1505G>A p.(Arg502Gln), MYBPC3 c.1468G>A p.(Gly490Arg)	
PT094	*Not genotyped*	
PT095	*Not genotyped*	
PT096	negative test	
PT097	MYBPC3 c.1020C>G p.(Tyr340*)	VUS
PT098	negative test	
PT099	MYH7 c.431G>A p.(Gly144Asp)	Pathogenic/likely pathogenic
PT100	negative test	

Symbols: *= the variant MYH7 c.2606G>A p.(Arg869His) is a founder effect in Tuscany, co-segregating in 16 different families, and is therefore considered pathogenic at our Center.

**= JPH2 c.505G>A (p.Glu169Lys) was identified in a sarcomere-negative patient who has been the object of a translational study reported in Beavers et al (Mutation E169K in Junctophilin-2 Causes Atrial Fibrillation Due to Impaired RyR2 Stabilization. J Am Coll Cardiol 2013; ;62:2010–9). This proband had an unusual clinical presentation with juvenile-onset paroxysmal AF (pAF) and marked QT prolongation. The proband's father, carrying the same mutation, also exhibited supraventricular tachycardia in addition to HCM. A pseudoknock-in mouse models was generated to determine the molecular defects underlying the development of arrhythmias caused by this JPH2 mutation. The mutation, failed to induce hypertrophy in the mouse but was found to account for the unusual occurrence of atrial fibrillation.

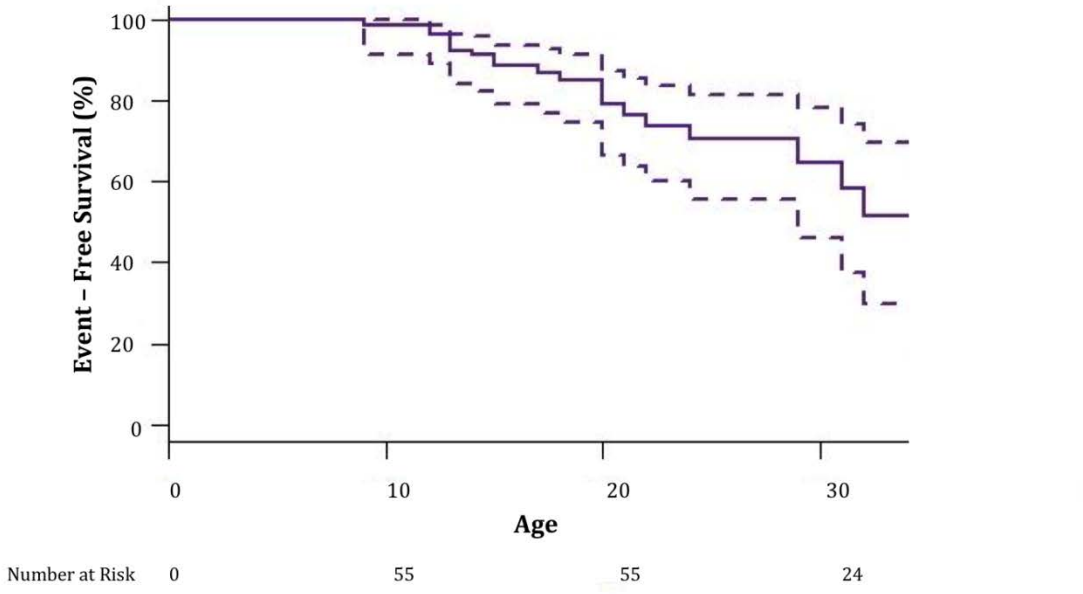
eTable 2. Performance of Clinical Risk Predictors for Arrhythmic Events.

Abbreviations: RF= risk factor; CI=confidence interval; HR= hazard ratio; LVOTO= left ventricular outflow tract obstruction; BP= blood pressure; PPV: Positive Predictive Value; NPV: Negative Predictive Value; other abbreviations as in Table 1.

	Risk Factor (Overall % of patients)	No LAE (n=81)	LAE (n=19)	P value**	Positive Predictive Value	Negative Predictive Value	Sensitivity (%)[CI]	Specificity (%)[CI]
Adult SCD Risk Factors*								
	Syncope (7%)	4 (5%)	3 (16%)	0.53	0.43	0.82	15 [3-39]	95 [87-98]
	Fx of SCD (19%)	17 (21%)	2 (11%)	0.24	0.11	0.79	10 [1-33]	79 [69-87]
	Non-sustained VT (13%)	8 (10%)	5 (26%)	0.15	0.38	0.83	26 [9-51]	90 [81-95]
	Extreme LVH (19%)	14 (17%)	5 (26%)	0.72	0.36	0.82	26 [9-51]	82 [72-90]
	LVOTO (16%)	12 (15%)	4 (21%)	0.35	0.25	0.82	21 [6-45]	85 [75-92]
	Inappropriate BP response (9%)	7 (9%)	2 (11%)	0.67	0.22	0.81	10 [1-33]	91 [83-96]
	0 RFs (46%)	40 (49%)	6 (32%)	0.62	0.13	0.76	32 [13-56]	51 [39-61]
	1 RF (44%)	35 (42%)	9 (47%)	0.79	0.20	0.82	47 [24-71]	57 [45-67]
	>1 RF (10%)	6 (9%)	4 (21%)	0.81	0.40	0.83	21 [6-45]	93 [84-97]
	ESC SCD Low Risk (74%)	65 (80%)	9 (47%)	0.02	0.40	0.81	67 [24-71]	75 [45-97]
	ESC SCD Intermediate Risk (18%)	11 (14%)	7(37%)	0.01	0.34	0.78	21 [9-45]	89 [84-97]
	ESC SCD High Risk (8%)	5 (6%)	3 (16%)	0.23	0.32	0.80	47 [21-61]	87 [45-67]
Functional Class								
	NYHA > I /Ross Score >2 (42%)	24 (29%)	18 (95%)	<0.01	0.43	0.98	95 [74-99]	70 [60-81]
Genetic Background								
	Thick Filament Mutations (66%)^	36 (44%)	8 (42%)	0.52	0.18	0.83	53 [27-78]	49 [36-60]
	Thin Filament Mutations (13%)^	2 (2%)	7 (37%)	<0.01	0.77	0.89	47 [21-73]	97 [90-99]
	Genotype Negative (21%)^	14 (19%)	0	0.03	0	0.79	0	79 [67-87]

eFigure 1- Long-Term Outcome of Pediatric-Onset HCM.

Risk of life-threatening cardiovascular events (A) in patients with pediatric-onset HCM spans from the second to the fourth decade of life, reaching its peak around age 20.



Abbreviations: MACE . Major Cardiovascular Event; LAE . life-threatening arrhythmic event; HF related . Heart Failure related event.