Lack of Phenotypic Differences by Cardiovascular Magnetic Resonance Imaging in MYH7 (β-Myosin Heavy Chain)-Versus MYBPC3 (Myosin-Binding Protein C)-Related Hypertrophic Cardiomyopathy

Adaya Weissler-Snir, MD*; Waseem Hindieh, MD*; Christiane Gruner, MD; Dana Fourey, MD; Evan Appelbaum, MD; Ethan Rowin, MD; Melanie Care, MSc; John R. Lesser, MD; Tammy S. Haas, RN; James E. Udelson, MD; Warren J. Manning, MD; Iacopo Olivotto, MD; Benedetta Tomberli, MD; Barry J. Maron, MD; Martin S. Maron, MD; Andrew M. Crean, MD; Harry Rakowski, MD; Raymond H. Chan, MD, MPH

Background—The 2 most commonly affected genes in hypertrophic cardiomyopathy (HCM) are MYH7 (β-myosin heavy chain) and MYBPC3 (β-myosin-binding protein C). Phenotypic differences between patients with mutations in these 2 genes have been inconsistent. Scarce data exist on the genotype–phenotype association as assessed by tomographic imaging using cardiac magnetic resonance imaging.

Methods and Results—Cardiac magnetic resonance imaging was performed on 358 consecutive genotyped hypertrophic cardiomyopathy probands at 5 tertiary hypertrophic cardiomyopathy centers. Genetic testing revealed a pathogenic mutation in 159 patients (44.4%). The most common genes identified were MYH7 (n=53) and MYBPC3 (n=75); 33.1% and 47% of genopositive patients, respectively. Phenotypic characteristics by cardiac magnetic resonance imaging of these 2 groups were similar, including left ventricular volumes, mass, maximal wall thickness, morphology, left atrial volume, and mitral valve leaflet lengths (all *P*=non-significant). The presence of late gadolinium enhancement (65% versus 64%; *P*=0.99) and the proportion of total left ventricular mass (%late gadolinium enhancement; 10.4±13.2% versus 8.5±8.5%; *P*=0.44) were also similar.

Conclusions—This multicenter multinational study shows lack of phenotypic differences between MYH7- and MYBPC3-associated hypertrophic cardiomyopathy when assessed by cardiac magnetic resonance imaging. Postmutational mechanisms appear more relevant to thick-filament disease expression and outcome than the disease-causing variant per se. **(*Circ Cardiovasc Imaging*. 2017;10:e005311. DOI: 10.1161/CIRCIMAGING.116.005311.)**

Key Words: cardiomyopathy, hypertrophic ■ genotype ■ phenotype ■ magnetic resonance imaging ■ myosin heavy chain ■ myosin-binding protein C

Hypertrophic cardiomyopathy (HCM) is the most commonly inherited monogenic cardiac disease with an estimated prevalence of 1:500.[1] HCM is inherited as an autosomal dominant trait with a variable expression and age-related penetrance.[2,3] In ≈35% to 60% of cases, HCM is caused by mutations in genes encoding sarcomere proteins.[4,5] To date, >1400 mutations in >13 genes have been identified, with the MYH7 (β-myosin heavy chain) and MYBPC3 (myosin-binding protein C) most commonly affected, accounting for >70% of genotyped families.[2,6]

See Editorial by Tower-Rader and Desai
See Clinical Perspective

Although thin-filament disease seems to have distinct phenotypic features in terms of left ventricular (LV) hypertrophy (LVH), cardiac magnetic resonance (CMR) late gadolinium enhancement (LGE) distribution, and diastolic abnormalities,[7] findings on the phenotypic differences between patients with *MYH7* and *MYBPC3* gene mutations have been inconsistent.

Received June 22, 2016; accepted December 19, 2016.

From the Division of Cardiology (A.W.-S., W.H., C.G., D.F., M.C., A.M.C., H.R., R.H.C.) and Joint Department of Medical Imaging (A.M.C.), University Health Network, Toronto, Ontario, Canada; Division of Cardiology, Cardiovascular Center, University Hospital Zurich, Switzerland (C.G.); Department of Medicine, Cardiovascular Division, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA (E.A., W.J.M., R.H.C.); Hypertrophic Cardiomyopathy Center, Tufts Medical Center, Boston, MA (E.R., J.E.U., B.J.M., M.S.M.); The Hypertrophic Cardiomyopathy Center, Minneapolis Heart Institute Foundation, MN (J.R.L., T.S.H.); and Referral Center for Cardiomyopathies, Careggi University Hospital, Florence, Italy (I.O., B.T.).

*Drs Weissler-Snir and Hindieh contributed equally to this work.

The Data Supplement is available at http://circimaging.ahajournals.org/lookup/suppl/doi:10.1161/CIRCIMAGING.116.005311/-/DC1.

Correspondence to Raymond H. Chan, MD, MPH, Division of Cardiology, University Health Network, 200 Elizabeth St, Toronto, Ontario M5G 2C4, Canada. E-mail raymond.chan@utoronto.ca

Although several small studies showed that *MYBPC3* gene mutations were associated with a more benign course and a later onset, other studies failed to show this association.[6,8–22]

CMR enables 3-dimensional tomographic assessment of cardiac anatomy and is considered the gold noninvasive standard for the evaluation of LV mass, volumes, and ejection fraction (LVEF). Additionally, LGE has the unique ability to identify areas of fibrosis.[23,24] Scarce data exist on the genotype–phenotype association in patients with HCM as assessed by CMR.[25] We sought to compare CMR phenotype between patients harboring mutations in the 2 most common genes, *MYH7* and *MYBPC3*.

Methods

Patient Selection

CMR was performed on 358 consecutive genotyped HCM probands who were enrolled as part of the multicenter registry involving 5 tertiary HCM centers in North America and Europe between November 2001 and February 2010.[26] HCM diagnosis was defined as a hypertrophied and nondilated LV (wall thickness ≥15 mm) in the absence of another cardiac or systemic disease expected to produce a similar magnitude of hypertrophy.[27,28] Only patients with a single pathogenic mutation in either *MYBPC3* or *MYH7* genes were included in the analysis. All patients gave informed consent before undergoing genetic testing. The study was approved by the Research Ethics Board at each respective institution.

Genotype

Genetic testing for HCM comprised a combination of commercially available panels, including oligonucleotide hybridization–based DNA sequencing and dideoxy-based DNA sequencing. Testing initiated after February 2008 assessed 8 HCM-associated myofilament-encoding genes, involving *MYBPC3*, *MYH7*, essential and regulatory myosin light chains, cardiac troponin T, cardiac troponin I, α-tropomyosin, and cardiac actin, as well as 3 genes associated with metabolic cardiomyopathies: *GLA* for Fabry disease, *LAMP2* for Danon disease, and *PRKAG2* for PRKAG2 cardiomyopathy. Testing initiated before February 2008 did not include the *GLA* gene, and testing was initiated only in the setting of a clinical presentation suggestive of Fabry disease. Gene variants detected by genetic testing that were classified as pathogenic or presumed pathogenic were considered disease causing (positive genotype). Variant pathogenicity was confirmed based on a central validation of mutations by a genetic counselor according to the most updated American College of Medical Genetics and Genomics standards and guidelines.[29] These determinations were derived from a combination of factors, which may have included absence in control chromosomes, presence in unrelated probands, in silico predictions, conservation across species, and segregation analysis. Variants of unknown significance were classified as a negative genotype because of ambiguity with respect to their pathogenic role (Table I in the Data Supplement).

CMR Analysis

CMR imaging was performed with a 1.5-T scanner using steady-state, free-precession breath-hold cines in 3 long-axis planes and sequential short-axis slices from the atrioventricular ring to the LV apex. LGE CMR imaging was performed 10 to 15 minutes after injection of 0.2 mmol/kg of gadolinium-diethylenetriaminepentaacetic acid with 2-dimensional segmented inversion recovery. Images from all centers were transferred to a core laboratory (PERFUSE, Boston, MA) for central, blinded analysis. LV volume, mass, and LVEF were measured by use of standard volumetric techniques and analyzed with commercially available software (QMASS version 7.4; Medis, Inc). LV endocardial and epicardial borders on cine images were manually planimetered to define the myocardium, taking care to exclude papillary muscles and the intertrabecular blood pool. Maximal LV wall thickness was defined as the greatest dimension at any site within the LV myocardium. LV chamber was assessed according to the American Heart Association 17-segment model.[30]

LGE was defined quantitatively by a myocardial postcontrast signal intensity 6 SD above that within a reference region of remote myocardium (without LGE) within the same slice and by visual assessment.[31,32] Regions with LGE were planimetered semiautomatically using QMASS 7.4 software. Total LGE mass was calculated by summing planimetered LGE areas and expressed as a proportion of LV myocardium (%LGE).

Anterior mitral leaflet and posterior mitral leaflet lengths were measured in diastole in the 3-chamber view as described by Maron et al.[33]

Statistical Analysis

Data are presented as mean±SD for normally distributed variables and as median (interquartile range) for non-normally distributed variables. Continuous variables were compared using Student *t* testing or Mann–Whitney testing, as appropriate. Categorical variables were compared using χ^2 statistics or Fischer exact testing, as appropriate. Cumulative event rates for outcomes including sudden death/appropriate shock, septal reduction, atrial fibrillation, and LVEF <50% were estimated using the Kaplan–Meier method and compared using log-rank statistics.

All tests were 2-tailed, with a $P<0.05$ considered significant.

Statistical analysis was performed using SAS for Windows (version 9.3; SAS, Cary, NC).

Results

Patients' Baseline Characteristics

Genetic testing revealed a pathogenic mutation in 159 patients (44.4%). The most common genes were MYH7 (n=53) and MYBPC3 (n=75); 33.1% and 47% of genopositive patients, respectively. Table 1 describes the patients' baseline characteristics, which were similar with the exception of the proportion of men among patients with *MYBPC3* mutations being significantly greater than among patients with *MYH7* gene mutations (65% versus 40%; $P=0.004$). The risk profile for sudden cardiac death (SCD) was similar between the 2 groups.

CMR Findings

Table 2 depicts the CMR findings of patients with *MYH7* gene mutation versus *MYBPC3* gene mutation. Both groups had similar LV volumes, mass, LVEF, and maximal wall thickness. The distribution of the different morphologies (reversed curvature, neutral, sigmoid, and apical) was also similar. The presence of LGE was similar in patients with *MYH7* gene mutation as compared with patients with *MYBPC3* gene mutation (65% versus 64%, $P=0.99$). There was no significant difference in LGE burden, when quantified either as absolute mass or indexed as percentage of LV mass.

Of note, in a subanalysis of patients aged ≥40 years (n=77), LVEF was lower in patients with *MYBPC3* gene mutations (n=45) as compared with *MYH7* gene mutations (n=32; 61.3% versus 65.5%, $P=0.046$). However, in contrast, there was no significant difference in quantified LGE in the 2 groups.

Outcomes

During a median follow-up time of 23.5 months (interquartile range, 14–36 months) there were no significant differences in outcomes between patients with *MYH7* and *MYBPC3* gene mutations including SCD (3.7% versus 1.3%; log-rank *P*=NS), septal reduction (13.2% versus 5.3%; log-rank *P*=NS), LVEF

Table 1. Patients' Baseline Characteristics According to Mutated Genes

Variable	MYBPC3+ (n=75)	MYH7+ (n=53)	*P* Value
Age, y	43.9±14.3	41.8±16.4	0.44
Male sex, n (%)	49 (65)	21 (40)	0.004
BSA	1.92±0.22	1.83±0.28	0.05
NYHA functional class I/II/III/IV, n (%)	44/21/8/2 (59/28/11/2.6)	26/20/7/0 (49/38/130/0)	0.38
Obstructive HCM*, n (%)	43 (57.4)	25 (46.4)	0.36
Atrial fibrillation, n (%)	10 (13)	10 (19)	0.40
Coronary artery disease, n (%)	1 (1.3)	2 (5.7)	0.31
Risk factors for SCD			
NSVT on 24–48 Holter monitor†, n (%)	19 (25)	9 (17)	0.26
Syncope, n ()	6 (8)	6 (11)	0.55
Family history of SCD, n (%)	25 (33)	17 (32)	0.88
Maximal wall thickness ≥ 30 mm, n (%)	6 (8)	3 (5.7)	0.74
Mean number of risk factors (SD)	0.75±0.79	0.66±0.68	0.52

Continuous variables are presented as mean±SD and categorical variables as percentages.

BSA indicates body surface area; HCM, hypertrophic cardiomyopathy; NSVT, nonsustained ventricular tachycardia; NYHA, New York Heart Association; and SCD, sudden cardiac death.

*Obstructive HCM was defined as left ventricular outflow tract gradient ≥30 mm Hg at rest or postprovocation on echocardiography.

†Nonsustained ventricular tachycardia was defined as ≥3 beats at ≥ 120 bpm.

Table 2. Findings on CMR Imaging According to Mutated Genes

Variable	MYBPC3+ (n=75)	MYH7+ (n= 53)	*P* Value
LVEDVi, mL/m^2	79.8±17.9	75.6±16.1	0.18
LVESVi, mL/m^2	27.2±11.2	30.4±11.6	0.12
LVEF, %	63.5±9.9	66.4±6.9	0.08
LVMi, g/m^2	76.7±27.2	71.6±26.2	0.28
LV maximal wall thickness, mm	20.6±5.4	20.6±6.1	0.99
LAVi, mL/m^2	58.1±27.5	53.4±24.5	0.32
LGE present, %	49 (65)	34 (64)	0.99
LGE mass by visual assessment, g	4.1 (0–13.2)	2.53 (0–7.2)	0.41
LGE mass ≥6 SD, g	1.79 (0–10.15)	1.21 (0–5.48)	0.68
Median %LGE of total LV mass by visual assessment (IQR)	2.2 (0–9.3)	1.7 (0–6.1)	0.69
Median ≥6 SD %LGE of total LV mass (IQR)	1.06 (0–7.64)	0.95 (0–4.62)	0.86
Anterior mitral valve leaflet, mm*	23.2±3.2	22.1±4.1	0.21
Posterior mitral valve leaflet, mm†	12.0±1.9	12.0±1.9	0.86
Morphology			0.83
Reversed curvature, n (%)	38 (50.7)	30 (56.6)	
Neutral septum, n (%)	19 (25.3)	14 (26.4)	
Sigmoid septum, n (%)	11 (14.7)	6 (11.3)	
Apical, n (%)	3 (4.0)	2 (3.8)	
Others, n (%)	4 (5.3)	1 (1.9)	

Continuous variables normally distributed are presented as mean±SD, non-normally distributed as median and IQR, and categorical variables as percentages.

CMR indicates cardiac magnetic resonance; IQR, interquartile range; LAVi, left atrial volume indexed; LGE, late gadolinium enhancement; LV, left ventricle; LVEDD, left ventricular end-diastolic diameter; LVEDVi, left ventricular end-diastolic volume indexed to body surface area; LVEF, left ventricular ejection fraction; LVESVi, left ventricular end-systolic volume indexed to body surface area; and LVMi, left ventricular mass indexed to body surface area.

*n=30 for *MYBPC3* (myosin-binding protein C) and 21 for *MYH7* (β-myosin heavy chain).

†n=45 for *MYBPC3* and 32 for *MYH7*.

<50% (3.8% versus 6.7%; log-rank *P*=NS), and atrial fibrillation (16.9% versus 18.7%; log-rank *P*=NS; Figures I and II in the Data Supplement).

Thin Filaments

In exploratory analyses, we examined the 31 patients with pathogenic thin-filament gene mutations. This group of patients when compared with patients with mutations in *MYBPC3* and *MYH7* genes had a significantly lower prevalence of LV outflow tract obstruction (32.2% versus 53.1%; *P*=0.045) and family history of SCD (9.7% versus 32.8%; *P*=0.01; Table II in the Data Supplement). On CMR, those patients harboring thin-filament gene mutations had a significantly lower LVEF, yet still in the normal range (59.6% versus 64.7%; *P*=0.007), with a higher indexed LV end-systolic volumes (35.7 versus 29 mL/m^2; *P*=0.036). Notably, there were no significant differences in the presence and proportion of LGE, magnitude of LVH, indexed LV mass, and morphology subtypes between patients with thick and thin-filament gene mutations (Table III in the Data Supplement).

Discussion

This is the largest study to date that compares the CMR phenotypic characteristics among HCM patients carrying the *MYH7* and *MYBPC3* gene mutations. In ≈45% of our cohort, similar to previous studies, genetic testing revealed a pathogenic mutation.[13,14,34,35] The most common variants were detected in the *MYBPC3* gene followed by *MYH7* gene, together accounting for more than 3 quarters of HCM variants detected. In contrast to the *MYH7* gene, where most mutations were missense mutations, about two thirds of mutations in the *MYBPC3* gene were frameshift mutations or mutations that result in premature stop codons and in-frame insertions or deletions or that affect splicing. This is consistent with other HCM cohorts.[13,18] We have found no differences in phenotype between patients with *MYH7* and *MYBPC3* gene mutations. Specifically, there were no significant differences in LV, mitral valve or left atrium parameters.

Most previous studies have suggested that patients with identified sarcomere mutations have more severe disease.[6,35] A recent study by Rubinshtein et al[36] (n=245) found that LGE by contrast-enhanced magnetic resonance imaging was more common (75%) in patients with a positive genetic test compared with those (53%) with a negative genetic test (*P*=0.001). Yet, studies scrutinizing the relationship between the different genes and clinical phenotype provide conflicting results (Table IV in the Data Supplement). A recent study by Ellims et al[25] comparing only 17 patients with *MYBPC3* gene mutations and 11 patients with *MYH7* gene mutations found no significant differences in LV dimensions, volumes, maximal wall thickness, and LGE burden. We have confirmed their findings in a much larger cohort. A recent meta-analysis found no difference in the clinical expression between the 2 genes in terms of age at presentation, family history of SCD, and severity of LVH.[6]

In contrast, a German-wide multicenter study of 97 genopositive patients with HCM and 69 genopositive patients with dilated cardiomyopathy found that patients with *MYBPC3* gene mutations (n=52) had a higher mean interventricular septal wall thickness when compared with patients with *MYH7* gene mutations (n=35). Interestingly, the study showed that both *MYH7* and *MYBPC3* gene mutations were associated with left atrial dilatation compared with genonegative patients; patients with *MYBPC3* gene mutations had larger atrial sizes compared with those with *MYH7* gene mutations.[13] This is in concordance with the present findings; the average indexed left atrial volume among patients with either mutation was higher than the normal reported values.[37] However, we found no difference in left atrial volume between the 2 genes.

We found no significant differences in clinical outcomes between patients with *MYH7* gene mutations versus *MYBPC3* gene mutations. However, it is noteworthy that the study included few outcomes and hence may be underpowered to detect significant differences in outcomes between the groups.

In the present study, we found no significant difference in LVEF between patients with *MYBPC3* gene mutations when compared with *MYH7* gene mutations. However, in patients ≥40 years of age, those with *MYBPC3* gene mutations had significantly lower LVEF than those with *MYH7* gene mutations. Our findings are consistent with a small study involving 27 patients with a founder mutation in *MYBPC3* gene wherein 6 patients (22%) developed LV dysfunction (LVEF <50%) after the fourth decade. Notably, 2 of these developed LV dilatation with LV wall thinning.[38] Whether these differences in LV systolic function between patients with *MYBPC3* and *MYH7* gene mutations after the age of 40 years are indeed of significance will need further study.

Scarce data exist on differences in the presence and burden of myocardial fibrosis between patients harboring *MYBPC3* and *MYH7* gene mutations. We have detected similar prevalence and extent of myocardial fibrosis in genotyped patients with HCM irrespective of the involved gene (ie, *MYH7* versus *MYBPC3*). These findings are consistent with a previous small sample sized study by Ellims et al,[25] of 28 patients (17 with *MYBPC3* gene mutations and 11 with *MYH7* gene mutations). In their study, in addition to LGE, T1 mapping did not differ between patients with *MYBPC3* and *MYH7* gene mutations. Furthermore, Olivotto et al[39] have recently shown no significant difference in the average myocardial blood flow after infusion of dipyridamole between patients with *MYH7* and *MYBPC3* gene mutations. Of note, they did not compare the prevalence and extent of myocardial fibrosis between the 2 groups. However, myocardial fibrosis is believed to result from bursts of silent and recurrent microvascular ischemia.[40,41] Interestingly, the cells involved in the process of microvascular remodeling are not known to express any of the sarcomere proteins mutated in HCM.[42] Hence, a direct effect of the mutated proteins on the microvascular remodeling process has not been demonstrated to date. It is possible that the remodeling process occurs as a response to other changes caused by the mutated proteins or that the abnormality of the developmental process of the microvasculature is present in HCM.[42]

Elongation of the mitral valve leaflets has been previously documented in gene-positive patients irrespective of LVH.[33] However, there have been no studies that have examined the variability in mitral valve leaflet lengths among the specific gene mutations. We have shown that there was no difference in both anterior and posterior mitral valve leaflet lengths between *MYH7* and *MYBPC3* gene mutations. The lack of differences in mitral valve leaflet length, severity of LVH, and type of septal morphology may account for the similar prevalence of LV outflow tract obstruction in the 2 groups.

Notably, in explanatory analyses, we have also observed no significant structural differences between patients harboring a thick versus thin-filament gene mutation, including maximal wall thickness and LV mass, presence and magnitude of LGE, and morphology subtypes. Although the mean LVEF of patients with thin-filament gene mutations was significantly lower than that of patients with thick-filament gene mutations, this was still in the normal range and hence should not have clinical implications. It is noteworthy that a recent study by Coppini et al[7] comparing the clinical phenotype and outcomes of 80 and 150 patients with thin and thick-filament gene mutations, respectively, found that patients with thin-filament gene mutations had a higher likelihood of advanced LV dysfunction and heart failure. In a subset of patients who had a CMR (≈60% of patients) those with thin-filament mutations had a lower LVEF. However, in contrast to our findings, patients with thin-filament gene mutations had a smaller LV mass index and greater LGE burden compared with patients with thick-filament gene mutation.[7]

The lack of phenotypic differences between the different genes and the great variability in clinical HCM expression even among families sharing identical mutations suggest that there are other epigenetic factors in addition to the disease-causing mutation, which affect phenotypic expression. Accordingly, in the era of whole-genome analysis, assessment of phenotypic expression may require analysis beyond single gene mutations, which may provide novel insight into the pathogenesis of the disorder. Modifier genes, which are neither necessary nor sufficient to cause the HCM phenotype, seem to exert an important role in HCM and affect disease expression. Several studies have demonstrated the role of the renin–angiotensin–aldosterone system in modifying the phenotype in HCM.

Ortlepp et al[43] have shown that genetic polymorphisms of the renin–angiotensin–aldosterone system influence

penetrance and degree of LVH in 26 patients from a single family with HCM caused by a *MYBPC3* mutation. Perkins et al[44] studied the role of 5 different renin–angiotensin–aldosterone system polymorphism genotypes in a cohort of 389 unrelated HCM patients genotyped for HCM-causing mutations in 8 sarcomeric/myofilament genes. They have demonstrated that renin–angiotensin–aldosterone system genotypes may modify the clinical phenotype of HCM in a disease gene-specific fashion rather than indiscriminately, where significant pro-LVH effect of deletion/deletion in the angiotensin-converting enzyme was present only in patients with MYBPC3 HCM. In a South Indian HCM cohort, Rangaraju et al[45] have found genetic variants in angiotensin-converting enzyme, heat shock protein, and tumor necrosis factor-α genes to be associated with increased LVH, LV outflow tract obstruction, and clinical symptoms. Another study by Wang et al[46] on a Chinese cohort of HCM patients has identified a correlation between rs2106809 polymorphism of angiotensin-converting enzyme-2 gene allele and LVH in male patients. Additionally, Brugada et al[47] have shown increased hypertrophy in HCM patients carrying endothelin-1 gene variants. Because HCM is more common among males, Lind et al[48] investigated whether an association exists between genetic variation in sex hormone receptors and the development of LVH in HCM. They have illustrated that variation at the androgen receptor gene is associated with LVH in men with HCM.

The identification of the disease-causing mutations in the sarcomere genes 2 decades ago have brought with it great hope that identification of a disease causative mutation in an individual could be used as a clinical tool for prediction of disease severity and risk stratification for SCD. However, our findings demonstrate that currently available genetic testing, which includes genes known to cause HCM, may provide only limited additional clinical information. In this study, there were only limited (if any) phenotypic differences between the 2 most commonly found genes. Although the study was underpowered to detect differences in outcomes given the low event rates, there do not seem to be any meaningful trends in event rates that may translate into significant differences in larger populations. Therefore, at present, information from genetic testing may not contribute significantly to risk stratification or alter clinical management in the majority of patients with HCM. Yet, it plays a pivotal role in HCM as it helps to confirm a molecular diagnosis in the proband and is an invaluable tool in cascade screening of at-risk family members.[27,28]

Limitations

Our study did not assess differences in penetrance among patients harboring *MYH7* and *MYBPC3* gene mutations as it included only patients who also had HCM phenotype. Second, we did not assess for modifier genes because only a few have been identified thus far. Finally, only patients who were able to undergo a CMR imaging were included; individuals with an implantable cardioverter defibrillator were not part of the analysis. Thus, we cannot exclude potential for selection bias.

Conclusions

The present study shows lack of differences in cardiac morphology between *MYH7* and *MYBPC3* gene mutations as assessed by CMR. Although identification of the specific thick-filament mutation does not seem to provide additional clinical information in HCM patients, detecting pathogenic gene variants remains useful in confirmation of diagnosis and cascade screening.

Disclosures

None.

References

1. Maron BJ, McKenna WJ, Danielson GK, Kappenberger LJ, Kuhn HJ, Seidman CE, Shah PM, Spencer WH, 3rd, Spirito P, Ten Cate FJ, Wigle ED, Task Force on Clinical Expert Consensus Documents. American College of Cardiology and Committee for Practice Guidelines. European Society of Cardiology. American College of Cardiology/European Society of Cardiology clinical expert consensus document on hypertrophic cardiomyopathy. A report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents and the European Society of Cardiology Committee for Practice Guidelines. *J Am Coll Cardiol*. 2003;42:1687–713.
2. Maron BJ, Maron MS, Semsarian C. Genetics of hypertrophic cardiomyopathy after 20 years: clinical perspectives. *J Am Coll Cardiol*. 2012;60:705–715. doi: 10.1016/j.jacc.2012.02.068.
3. Seidman CE, Seidman JG. Identifying sarcomere gene mutations in hypertrophic cardiomyopathy: a personal history. *Circ Res*. 2011;108:743–750. doi: 10.1161/CIRCRESAHA.110.223834.
4. Elliott P, McKenna WJ. Hypertrophic cardiomyopathy. *Lancet*. 2004;363:1881–1891. doi: 10.1016/S0140-6736(04)16358-7.
5. Seidman JG, Seidman C. The genetic basis for cardiomyopathy: from mutation identification to mechanistic paradigms. *Cell*. 2001;104:557–567.
6. Lopes LR, Rahman MS, Elliott PM. A systematic review and meta-analysis of genotype-phenotype associations in patients with hypertrophic cardiomyopathy caused by sarcomeric protein mutations. *Heart*. 2013;99:1800–1811. doi: 10.1136/heartjnl-2013-303939.
7. Coppini R, Ho CY, Ashley E, Day S, Ferrantini C, Girolami F, Tomberli B, Bardi S, Torricelli F, Cecchi F, Mugelli A, Poggesi C, Tardiff J, Olivotto I. Clinical phenotype and outcome of hypertrophic cardiomyopathy associated with thin-filament gene mutations. *J Am Coll Cardiol*. 2014;64:2589–2600. doi: 10.1016/j.jacc.2014.09.059.
8. Charron P, Dubourg O, Desnos M, Bennaceur M, Carrier L, Camproux AC, Isnard R, Hagege A, Langlard JM, Bonne G, Richard P, Hainque B, Bouhour JB, Schwartz K, Komajda M. Clinical features and prognostic implications of familial hypertrophic cardiomyopathy related to the cardiac myosin-binding protein C gene. *Circulation*. 1998;97:2230–2236.
9. Maron BJ, Niimura H, Casey SA, Soper MK, Wright GB, Seidman JG, Seidman CE. Development of left ventricular hypertrophy in adults in hypertrophic cardiomyopathy caused by cardiac myosin-binding protein C gene mutations. *J Am Coll Cardiol*. 2001;38:315–321.
10. Marian AJ, Mares A Jr, Kelly DP, Yu QT, Abchee AB, Hill R, Roberts R. Sudden cardiac death in hypertrophic cardiomyopathy. Variability in phenotypic expression of beta-myosin heavy chain mutations. *Eur Heart J*. 1995;16:368–376.
11. Kokado H, Shimizu M, Yoshio H, Ino H, Okeie K, Emoto Y, Matsuyama T, Yamaguchi M, Yasuda T, Fujino N, Ito H, Mabuchi H. Clinical features of hypertrophic cardiomyopathy caused by a Lys183 deletion mutation in the cardiac troponin I gene. *Circulation*. 2000;102:663–669.
12. Ackerman MJ, VanDriest SL, Ommen SR, Will ML, Nishimura RA, Tajik AJ, Gersh BJ. Prevalence and age-dependence of malignant mutations in the beta-myosin heavy chain and troponin T genes in hypertrophic cardiomyopathy: a comprehensive outpatient perspective. *J Am Coll Cardiol*. 2002;39:2042–2048.
13. Waldmüller S, Erdmann J, Binner P, Gelbrich G, Pankuweit S, Geier C, Timmermann B, Haremza J, Perrot A, Scheer S, Wachter R, Schulze-Waltrup N, Dermintzoglou A, Schönberger J, Zeh W, Jurmann B, Brodherr T, Börgel J, Farr M, Milting H, Blankenfeldt W, Reinhardt R, Özcelik C, Osterziel KJ, Loeffler M, Maisch B, Regitz-Zagrosek V, Schunkert H, Scheffold T; German Competence Network Heart Failure. Novel correlations between the genotype and the phenotype of hypertrophic and dilated cardiomyopathy: results from the German Competence Network Heart Failure. *Eur J Heart Fail*. 2011;13:1185–1192. doi: 10.1093/eurjhf/hfr074.
14. Millat G, Bouvagnet P, Chevalier P, Dauphin C, Jouk PS, Da Costa A, Prieur F, Bresson JL, Faivre L, Eicher JC, Chassaing N, Crehalet H,

Porcher R, Rodriguez-Lafrasse C, Rousson R. Prevalence and spectrum of mutations in a cohort of 192 unrelated patients with hypertrophic cardiomyopathy. *Eur J Med Genet*. 2010;53:261–267. doi: 10.1016/j.ejmg.2010.07.007.
15. Wang S, Zou Y, Fu C, Xu X, Wang J, Song L, Wang H, Chen J, Wang J, Huan T, Hui R. Worse prognosis with gene mutations of beta-myosin heavy chain than myosin-binding protein C in Chinese patients with hypertrophic cardiomyopathy. *Clin Cardiol*. 2008;31:114–118. doi: 10.1002/clc.20151.
16. Van Driest SL, Vasile VC, Ommen SR, Will ML, Tajik AJ, Gersh BJ, Ackerman MJ. Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2004;44:1903–1910. doi: 10.1016/j.jacc.2004.07.045.
17. Van Driest SL, Jaeger MA, Ommen SR, Will ML, Gersh BJ, Tajik AJ, Ackerman MJ. Comprehensive analysis of the beta-myosin heavy chain gene in 389 unrelated patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2004;44:602–610. doi: 10.1016/j.jacc.2004.04.039.
18. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, Benaiche A, Isnard R, Dubourg O, Burban M, Gueffet JP, Millaire A, Desnos M, Schwartz K, Hainque B, Komajda M; EUROGENE Heart Failure Project. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation*. 2003;107:2227–2232. doi: 10.1161/01.CIR.0000066323.15244.54.
19. Charron P, Dubourg O, Desnos M, Isnard R, Hagege A, Bonne G, Carrier L, Tesson F, Bouhour JB, Buzzi JC, Feingold J, Schwartz K, Komajda M. Genotype-phenotype correlations in familial hypertrophic cardiomyopathy. A comparison between mutations in the cardiac protein-C and the beta-myosin heavy chain genes. *Eur Heart J*. 1998;19:139–145.
20. Page SP, Kounas S, Syrris P, Christiansen M, Frank-Hansen R, Andersen PS, Elliott PM, McKenna WJ. Cardiac myosin binding protein-C mutations in families with hypertrophic cardiomyopathy: disease expression in relation to age, gender, and long term outcome. *Circ Cardiovasc Genet*. 2012;5:156–166. doi: 10.1161/CIRCGENETICS.111.960831.
21. García-Castro M, Coto E, Reguero JR, Berrazueta JR, Alvarez V, Alonso B, Sainz R, Martín M, Morís C. [Mutations in sarcomeric genes MYH7, MYBPC3, TNNT2, TNNI3, and TPM1 in patients with hypertrophic cardiomyopathy]. *Rev Esp Cardiol*. 2009;62:48–56.
22. Song L, Zou Y, Wang J, Wang Z, Zhen Y, Lou K, Zhang Q, Wang X, Wang H, Li J, Hui R. Mutations profile in Chinese patients with hypertrophic cardiomyopathy. *Clin Chim Acta*. 2005;351:209–216. doi: 10.1016/j.cccn.2004.09.016.
23. Moon JC, Reed E, Sheppard MN, Elkington AG, Ho SY, Burke M, Petrou M, Pennell DJ. The histologic basis of late gadolinium enhancement cardiovascular magnetic resonance in hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2004;43:2260–2264. doi: 10.1016/j.jacc.2004.03.035.
24. Moravsky G, Ofek E, Rakowski H, Butany J, Williams L, Ralph-Edwards A, Wintersperger BJ, Crean A. Myocardial fibrosis in hypertrophic cardiomyopathy: accurate reflection of histopathological findings by CMR. *JACC Cardiovasc Imaging*. 2013;6:587–596. doi: 10.1016/j.jcmg.2012.09.018.
25. Ellims AH, Iles LM, Ling LH, Chong B, Macciocca I, Slavin GS, Hare JL, Kaye DM, Marasco SF, McLean CA, James PA, du Sart D, Taylor AJ. A comprehensive evaluation of myocardial fibrosis in hypertrophic cardiomyopathy with cardiac magnetic resonance imaging: linking genotype with fibrotic phenotype. *Eur Heart J Cardiovasc Imaging*. 2014;15:1108–1116. doi: 10.1093/ehjci/jeu077.
26. Chan RH, Maron BJ, Olivotto I, Pencina MJ, Assenza GE, Haas T, Lesser JR, Gruner C, Crean AM, Rakowski H, Udelson JE, Rowin E, Lombardi M, Cecchi F, Tomberli B, Spirito P, Formisano F, Biagini E, Rapezzi C, De Cecco CN, Autore C, Cook EF, Hong SN, Gibson CM, Manning WJ, Appelbaum E, Maron MS. Prognostic value of quantitative contrast-enhanced cardiovascular magnetic resonance for the evaluation of sudden death risk in patients with hypertrophic cardiomyopathy. *Circulation*. 2014;130:484–495. doi: 10.1161/CIRCULATIONAHA.113.007094.
27. Gersh BJ, Maron BJ, Bonow RO, Dearani JA, Fifer MA, Link MS, Naidu SS, Nishimura RA, Ommen SR, Rakowski H, Seidman CE, Towbin JA, Udelson JE, Yancy CW; American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. 2011 ACCF/AHA Guideline for the Diagnosis and Treatment of Hypertrophic Cardiomyopathy: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Developed in collaboration with the American Association for Thoracic Surgery, American Society of Echocardiography, American Society of Nuclear Cardiology, Heart Failure Society of America, Heart Rhythm Society, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. *Circulation*. 2011;124:e783–e831. doi: 10.1161/CIR.0b013e318223e2bd.
28. Authors/Task Force Members, Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, Charron P, Hagege AA, Lafont A, Limongelli G, Mahrholdt H, McKenna WJ, Mogensen J, Nihoyannopoulos P, Nistri S, Pieper PG, Pieske B, Rapezzi C, Rutten FH, Tillmanns C, Watkins H. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy: the Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). *Eur Heart J*. 2014;35:2733–2779. doi: 10.1093/eurheartj/ehu284.
29. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424. doi: 10.1038/gim.2015.30.
30. Fattori R, Biagini E, Lorenzini M, Buttazzi K, Lovato L, Rapezzi C. Significance of magnetic resonance imaging in apical hypertrophic cardiomyopathy. *Am J Cardiol*. 2010;105:1592–1596. doi: 10.1016/j.amjcard.2010.01.020.
31. Harrigan CJ, Peters DC, Gibson CM, Maron BJ, Manning WJ, Maron MS, Appelbaum E. Hypertrophic cardiomyopathy: quantification of late gadolinium enhancement with contrast-enhanced cardiovascular MR imaging. *Radiology*. 2011;258:128–133. doi: 10.1148/radiol.10090526.
32. Maron MS. Clinical utility of cardiovascular magnetic resonance in hypertrophic cardiomyopathy. *J Cardiovasc Magn Reson*. 2012;14:13. doi: 10.1186/1532-429X-14-13.
33. Maron MS, Olivotto I, Harrigan C, Appelbaum E, Gibson CM, Lesser JR, Haas TS, Udelson JE, Manning WJ, Maron BJ. Mitral valve abnormalities identified by cardiovascular magnetic resonance represent a primary phenotypic expression of hypertrophic cardiomyopathy. *Circulation*. 2011;124:40–47. doi: 10.1161/CIRCULATIONAHA.110.985812.
34. Van Driest SL, Ommen SR, Tajik AJ, Gersh BJ, Ackerman MJ. Sarcomeric genotyping in hypertrophic cardiomyopathy. *Mayo Clin Proc*. 2005;80:463–469. doi: 10.1016/S0025-6196(11)63196-0.
35. Olivotto I, Girolami F, Ackerman MJ, Nistri S, Bos JM, Zachara E, Ommen SR, Theis JL, Vaubel RA, Re F, Armentano C, Poggesi C, Torricelli F, Cecchi F. Myofilament protein gene mutation screening and outcome of patients with hypertrophic cardiomyopathy. *Mayo Clin Proc*. 2008;83:630–638. doi: 10.4065/83.6.630.
36. Rubinshtein R, Glockner JF, Ommen SR, Araoz PA, Ackerman MJ, Sorajja P, Bos JM, Tajik AJ, Valeti US, Nishimura RA, Gersh BJ. Characteristics and clinical significance of late gadolinium enhancement by contrast-enhanced magnetic resonance imaging in patients with hypertrophic cardiomyopathy. *Circ Heart Fail*. 2010;3:51–58. doi: 10.1161/CIRCHEARTFAILURE.109.854026.
37. Le Ven F, Bibeau K, De Larochellière É, Tizón-Marcos H, Deneault-Bissonnette S, Pibarot P, Deschepper CF, Larose É. Cardiac morphology and function reference values derived from a large subset of healthy young Caucasian adults by magnetic resonance imaging. *Eur Heart J Cardiovasc Imaging*. 2016;17:981–990. doi: 10.1093/ehjci/jev217.
38. Calore C, De Bortoli M, Romualdi C, Lorenzon A, Angelini A, Basso C, Thiene G, Iliceto S, Rampazzo A, Melacini P. A founder MYBPC3 mutation results in HCM with a high risk of sudden death after the fourth decade of life. *J Med Genet*. 2015;52:338–347. doi: 10.1136/jmedgenet-2014-102923.
39. Olivotto I, Girolami F, Sciagrà R, Ackerman MJ, Sotgia B, Bos JM, Nistri S, Sgalambro A, Grifoni C, Torricelli F, Camici PG, Cecchi F. Microvascular function is selectively impaired in patients with hypertrophic cardiomyopathy and sarcomere myofilament gene mutations. *J Am Coll Cardiol*. 2011;58:839–848. doi: 10.1016/j.jacc.2011.05.018.
40. Basso C, Thiene G, Corrado D, Buja G, Melacini P, Nava A. Hypertrophic cardiomyopathy and sudden death in the young: pathologic evidence of myocardial ischemia. *Hum Pathol*. 2000;31:988–998. doi: 10.1053/hupa.2000.16659.
41. Shirani J, Pick R, Roberts WC, Maron BJ. Morphology and significance of the left ventricular collagen network in young patients with hypertrophic cardiomyopathy and sudden cardiac death. *J Am Coll Cardiol*. 2000;35:36–44.
42. Olivotto I, Cecchi F, Poggesi C, Yacoub MH. Developmental origins of hypertrophic cardiomyopathy phenotypes: a unifying hypothesis. *Nat Rev Cardiol*. 2009;6:317–321. doi: 10.1038/nrcardio.2009.9.
43. Ortlepp JR, Vosberg HP, Reith S, Ohme F, Mahon NG, Schröder D, Klues HG, Hanrath P, McKenna WJ. Genetic polymorphisms in the

renin-angiotensin-aldosterone system associated with expression of left ventricular hypertrophy in hypertrophic cardiomyopathy: a study of five polymorphic genes in a family with a disease causing mutation in the myosin binding protein C gene. *Heart*. 2002;87:270–275.
44. Perkins MJ, Van Driest SL, Ellsworth EG, Will ML, Gersh BJ, Ommen SR, Ackerman MJ. Gene-specific modifying effects of pro-LVH polymorphisms involving the renin-angiotensin-aldosterone system among 389 unrelated patients with hypertrophic cardiomyopathy. *Eur Heart J*. 2005;26:2457–2462. doi: 10.1093/eurheartj/ehi438.
45. Rangaraju A, Rani DS, Satyanarayana M, Calambur N, Swapna N, Nallari P. Genetic variations of α-cardiac actin and cardiac muscle LIM protein in hypertrophic cardiomyopathy in South India. *Exp Clin Cardiol*. 2012;17:26–29.
46. Wang SX, Fu CY, Zou YB, Wang H, Shi Y, Xu XQ, Chen JZ, Song XD, Huan TJ, Hui RT. Polymorphisms of angiotensin-converting enzyme 2 gene associated with magnitude of left ventricular hypertrophy in male patients with hypertrophic cardiomyopathy. *Chin Med J (Engl)*. 2008;121:27–31.
47. Brugada R, Kelsey W, Lechin M, Zhao G, Yu QT, Zoghbi W, Quinones M, Elstein E, Omran A, Rakowski H, Wigle D, Liew CC, Sole M, Roberts R, Marian AJ. Role of candidate modifier genes on the phenotypic expression of hypertrophy in patients with hypertrophic cardiomyopathy. *J Investig Med*. 1997;45:542–551.
48. Lind JM, Chiu C, Ingles J, Yeates L, Humphries SE, Heather AK, Semsarian C. Sex hormone receptor gene variation associated with phenotype in male hypertrophic cardiomyopathy patients. *J Mol Cell Cardiol*. 2008;45:217–222. doi: 10.1016/j.yjmcc.2008.05.016.

CLINICAL PERSPECTIVE

The 2 most commonly affected genes in hypertrophic cardiomyopathy are MYH7 (β-myosin heavy chain) and MYBPC3 (β-myosin-binding protein C). Phenotypic differences between patients with mutations in these 2 genes have been inconsistent. Furthermore, scarce data exist on the genotype–phenotype association as assessed by tomographic imaging using cardiac magnetic resonance imaging. In the present study, cardiac magnetic resonance imaging was performed on 358 consecutive genotyped hypertrophic cardiomyopathy probands at 5 tertiary hypertrophic cardiomyopathy centers. Genetic testing revealed a pathogenic mutation in 159 patients (44.4%). The most common genes identified were *MYH7* (n=53) and *MYBPC3* (n=75); 33.1% and 47% of genopositive patients, respectively. Phenotypic characteristics by cardiac magnetic resonance imaging of these 2 groups were similar including left ventricular volumes, mass, maximal wall thickness, morphology, left atrial volume, and mitral valve leaflet lengths (all *P*=non-significant). The presence of late gadolinium enhancement (65% versus 64%; *P*=0.99) and the proportion of total left ventricular mass (% late gadolinium enhancement; 10.4±13.2% versus 8.5±8.5%; *P*=0.44) were also similar. In conclusion, this multicenter multinational study shows lack of differences in cardiac morphology between *MYH7* and *MYBPC3* gene mutations as assessed by cardiac magnetic resonance imaging. Although identification of the specific thick-filament mutation does not seem to provide additional clinical information in hypertrophic cardiomyopathy patients, detecting pathogenic gene variants remains useful in confirmation of diagnosis and cascade screening.

Circulation
Cardiovascular Imaging

Lack of Phenotypic Differences by Cardiovascular Magnetic Resonance Imaging in MYH7 (β-Myosin Heavy Chain)- Versus MYBPC3 (Myosin-Binding Protein C)-Related Hypertrophic Cardiomyopathy

Adaya Weissler-Snir, Waseem Hindieh, Christiane Gruner, Dana Fourey, Evan Appelbaum, Ethan Rowin, Melanie Care, John R. Lesser, Tammy S. Haas, James E. Udelson, Warren J. Manning, Iacopo Olivotto, Benedetta Tomberli, Barry J. Maron, Martin S. Maron, Andrew M. Crean, Harry Rakowski and Raymond H. Chan

Circ Cardiovasc Imaging. 2017;10:
doi: 10.1161/CIRCIMAGING.116.005311
Circulation: Cardiovascular Imaging is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1941-9651. Online ISSN: 1942-0080

Supplemental Material

Supplementary Table 1. Identified mutations by gene

No. of patients	Gene	Consequence	Mutation type	Classification
2	MYBPC3	c.3330+2T>G	Splice site	Pathogenic
3	MYBPC3	p.Pro1157fs	Frameshift	Pathogenic
1	MYBPC3	p.Lys666fs	Frameshift	Likely pathogenic
1	MYBPC3	c.3490+1G>A	Splice site	Pathogenic
1	MYBPC3	c.3330+5G>A	Intron variant	Pathogenic
2	MYBPC3	c.821+1G>A	Splice site	Pathogenic
12	MYBPC3	p.Glu258Lys	Missense	Pathogenic
3	MYBPC3	p.Arg502Gln	Missense	Pathogenic
4	MYBPC3	p.Tyr340Ter	Nonsense	Likely pathogenic
3	MYBPC3	p.Lys1065fs	Frameshift	Pathogenic
2	MYBPC3	p.Gly897fs	Frameshift	Likely pathogenic
1	MYBPC3	p.Tyr842Ter	Nonsense	Pathogenic
3	MYBPC3	p.Ala392fs	Frameshift	Likely pathogenic
2	MYBPC3	p.Thr1256del	In frame variant	Likely pathogenic
1	MYBPC3	p.Glu542Gln	Missense	Pathogenic
1	MYBPC3	p.Gly1206fs	Frameshift	Likely pathogenic
1	MYBPC3	p.Glu334Lys	Missense	Likely pathogenic
1	MYBPC3	p.Trp729fs	Frameshift	Likely pathogenic
1	MYBPC3	p.Asp1076fs	Frameshift	Likely pathogenic
1	MYBPC3	p.Phe1159fs	Frameshift	Likely pathogenic
1	MYBPC3	p.Val771fs	Frameshift	pathogenic
1	MYBPC3	c.3330+5G>A	Splice site	Pathogenic
1	MYBPC3	p.Ala1056fs	Frameshift	Likely pathogenic
2	MYBPC3	p.Arg502Trp	Missense	Pathogenic
1	MYBPC3	p.Pro453fs	Frameshift	Likely pathogenic
1	MYBPC3	c.1928-2A>G	Splice site	Pathogenic
1	MYBPC3	p.Tyr847Ter	Nonsense	Pathogenic
2	MYBPC3	p.Glu728Ter	Nonsense	Pathogenic
2	MYBPC3	p.Trp792fs	Frameshift	Pathogenic
1	MYBPC3	c.506-1G>C	Splice site	Likely pathogenic
1	MYBPC3	p.Glu1096Ter	Nonsense	Pathogenic
1	MYBPC3	p.Gly144fs	Frameshift	Likely pathogenic
1	MYBPC3	p.Pro955fs	Frameshift	Pathogenic
1	MYBPC3	p.Ile736Thr	Missense	Pathogenic
1	MYBPC3	p.Asn143Arg	Missense	Likely pathogenic
1	MYBPC3	p.Gly531Arg	Missense	Likely pathogenic
1	MYBPC3	p.Met690Thr	Missense	Pathogenic
1	MYBPC3	c.3628-41_3628-	Intron variant	Likely pathogenic

		17del25		
1	MYBPC3	p.Val1219Leu	Missense	Likely pathogenic
1	MYBPC3	p.Val840fs	Frameshift	Pathogenic
1	MYBPC3	c.1928-2A>G	Splice site	Pathogenic
1	MYBPC3	p.Pro944fs	Frameshift	Pathogenic
1	MYBPC3	c.3330+5G>C	Splice site	Pathogenic
1	MYBPC3	c.2905+1G>A	Splice site	Pathogenic
1	MYBPC3	c.1624+4A>T	Splice site	Likely pathogenic
1	MYBPC3	c.927-9G>A	Intron variant	Pathogenic
1	MYH7	p.Arg663Cys	Missense	Pathogenic
1	MYH7	p.Arg1712Gln	Missense	Likely pathogenic
4	MYH7	p.Asp906Gly	Missense	Pathogenic
1	MYH7	p.Arg719Gln	Missense	Pathogenic
1	MYH7	p.Gly733Val	Missense	Likely pathogenic
1	MYH7	p.Gly716Arg	Missense	Pathogenic
1	MYH7	p.Glu536Asp	Missense	Likely pathogenic
1	MYH7	p.Lys847Glu	Missense	Likely pathogenic
1	MYH7	p.Arg143Trp	Missense	Likely pathogenic
2	MYH7	p.Val606Met	Missense	Pathogenic
4	MYH7	p.Glu927Lys	Missense	Likely pathogenic
1	MYH7	p.Thr70Ser	Missense	Likely pathogenic
2	MYH7	p.Arg453His	Missense	Likely pathogenic
1	MYH7	p.Arg403Gln	Missense	Pathogenic
1	MYH7	p.Arg723Cys	Missense	Pathogenic
1	MYH7	p.Arg1712Gln	Missense	Likely pathogenic
1	MYH7	p.Glu894Gly	Missense	Likely pathogenic
4	MYH7	p.Gly584Arg	Missense	Likely pathogenic
2	MYH7	p.Glu497Asp	Missense	Likely pathogenic
3	MYH7	p.Glu1356Lys	Missense	Likely pathogenic
1	MYH7	p.Met982Thr	Missense	Likely pathogenic
6	MYH7	p.Leu908Val	Missense	Pathogenic
1	MYH7	p.Glu930Lys	Missense	Pathogenic
1	MYH7	p.Arg453Cys	Missense	Pathogenic
1	MYH7	p.Arg943Ter	Nonsense	Pathogenic
1	MYH7	p.Lys1216fs	Frameshift	Likely pathogenic
1	MYH7	p.Arg870His	Missense	Pathogenic
1	MYH7	p.Arg169Cys	Missense	Likely pathogenic
1	MYH7	p.Asp928Asn	Missense	Likely pathogenic
2	MYH7	p.Arg663His	Missense	Likely pathogenic
1	MYH7	p.Lys185fs	Frameshift	Likely pathogenic
1	MYH7	p.Ile263Thr	Missense	Pathogenic
1	MYH7	p.Ser444fs	Frameshift	Pathogenic
2	TPM1	p.Asp175Asn	Missense	Pathogenic
4	TPM1	p.Glu192Lys	Missense	Likely pathogenic
1	TNNC1	p.Gln122fs	Frameshift	Pathogenic
1	TNNI2	p.Ala157Val	Missense	Pathogenic
1	TNNI3	p.Arg192His	Missense	Pathogenic
1	TNNI3	p.Arg141Gln	Missense	Pathogenic

2	TNNI3	p.Arg145Gln	Missense	Likely pathogenic
2	TNNI3	p.Arg145Trp	Missense	Pathogenic
1	TNNI3	p.Ser199Asn	Missense	Likely pathogenic
2	TNNI3	p.Ala157Val	Missense	Pathogenic
1	TNNI3	c.549G>T	Splice site	Pathogenic
1	TNNT2	p.Ala104Val	Missense	Likely pathogenic
1	TNNT2	p.Arg130Cys	Missense	Likely pathogenic
5	TNNT2	p.Arg278Cys	Missense	Likely pathogenic
3	TNNT2	p.Glu163del	In frame deletion	Pathogenic
1	ACTC1	p.Glu101Lys	Missense	Pathogenic
1	MYL3	p.Ala57Gly	Missense	Pathogenic
1	MYL3	p.Asp178Asn	Missense	Pathogenic

Supplementary Table 2. Baseline characteristics of patients with thick versus thin filament gene mutations

Variable	Thick filaments (n=128)	Thin filaments (n=31)	P value
Age (years)	43.1±15.2	45.8±15.4	0.44
Male, n (%)	75 (58.6%)	18 (58.1%)	0.93
BSA	1.9±0.25	2.0±0.26	0.02
NYHA functional class I/II/III/IV, n (%)	60/41/15/2 (46.9/32/11.7/1.6%)	16/7/8/0 (51.6/22.6/25.8/0)	0.19
Obstructive HCM†, n (%)	68 (53.1)	10 (32.2)	0.045
Atrial fibrillation, n (%)	20 (15.6)	7 (22.6)	0.42
Coronary artery disease, n (%)	3 (2.3%)	1 (3.2%)	0.58
Risk factors for SCD			
NSVT on 24-	28 (21.9)	6 (19.3)	1.00

48 Holter monitor*, n (%)			
Syncope, n (%)	12 (9.4)	3 (9.7)	1.00
Family history of SCD, n (%)	42 (32.8)	3 (9.7)	0.01
Maximal wall thickness ≥ 30mm, n (%)	8 (6.2%)	0 (0%)	0.32

† Obstructive HCM was defined as left ventricular outflow tract gradient ≥30mmHg at rest or post provocation on echocardiography

* Nonsustained ventricular tachycardia was defined as ≥ 3 beats at ≥ 120bpm

Continuous variables are presented as mean ± standard deviation and categorical variables as percentages.

BSA=Body surface area; CMR=Cardiac magnetic resonance imaging; NSVT=Non sustained ventricular tachycardia; NYHA=New-York heart association; SCD=sudden cardiac death

Supplementary Table 3. Findings on cardiac MRI of patients with thick versus thin filament gene mutations

Variable	Thick filaments (n=128)	Thin filament (n=31)	P value
LVEDVi (ml/m2)	78.1±17.2	80.3±21.7	0.56
LVESVi (ml/m2)	29±13.7	35.7±14.2	0.036
LVEF (%)	64.7±8.9	59.6±8.2	0.007
Median LVMi (g/m2)	71.0 (54.5-87.4)	78.8 (61.6-112.5)	0.12
LV maximal wall thickness (mm)	20.6 ± 5.7	19.2±5.5	0.24
LGE present (%)	45 (35.1)	9 (29.0)	0.47
LGE mass by visual (g)	3.2 (0-11.3)	6.4 (0-19.9)	0.13
LGE mass ≥ 6 S.D. (g)	1.6 (0-6.24)	4.7 (0-15.23)	0.16
Median % LGE of total LV mass by visual assessment (IQR)	2.0 (1.1-4.2)	3.9 (0-14.7)	0.26
Median ≥ 6 S.D. % LGE of total LV mass, (IQR)	1.05 (0-5.2)	3.36 (0-9.9)	0.31
Anterior mitral valve leaflet, (mm)	22.7±3.6	24.4±3.0	0.037
Posterior mitral valve leaflet, (mm)	12.0±1.8	12.4±2.3	0.32

Morphology			0.16
Reversed curvature, n (%)	68 (53.1)	11 (35.5)	
Neutral septum, n (%)	33 (25.8)	8 (25.8)	
Sigmoid septum, n (%)	17 (13.3)	7 (22.6)	
Apical, n (%)	5 (3.9)	4 (12.9)	
Others, n (%)	5 (3.9)	1 (3.2)	

Continuous variables normally distributed are presented as mean ± standard deviation, non-normally distributed as median and interquartile range and categorical variables as percentages.

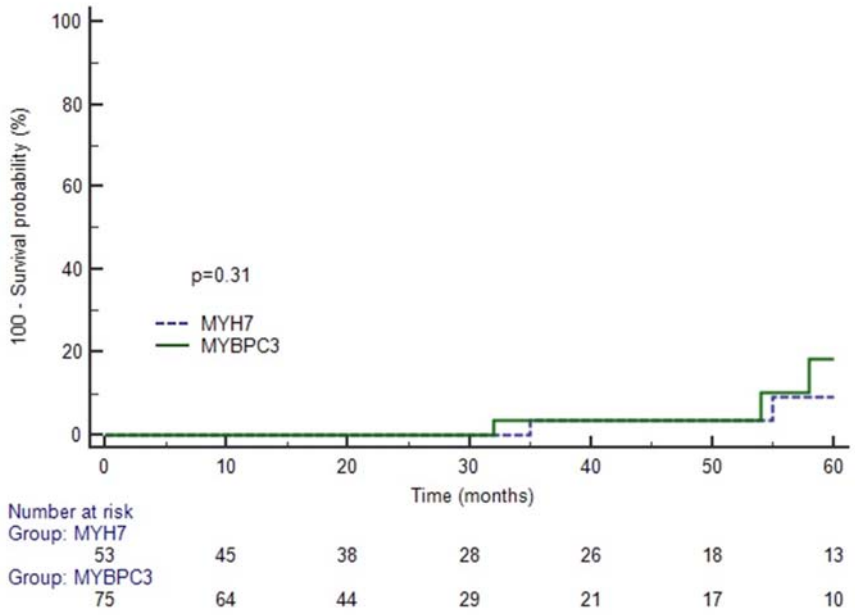
IQR = Interquartile range; LGE =Late gadolinium enhancement; LV=Left ventricle; LVEF=Left ventricular ejection fraction; LVEDD=LV end diastolic diameter; LVEDVi=LV end diastolic volume indexed to body surface area; LVESVi=LV end systolic volume indexed to body surface area; LVMi=LV mass indexed to body surface area; SD= standard deviation

Supplementary table 4. Previous studies comparing differences in phenotypic expression between patients with MYBPC3 mutations versus MYH7 mutations

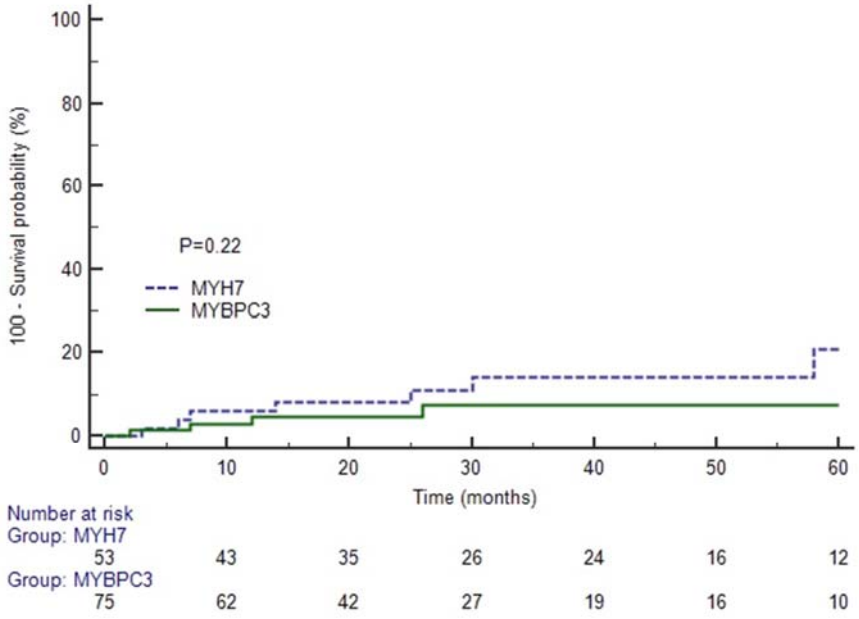
Studies demonstrating clinical differences between MYBPC3 and MYH7 mutations	
Biagini et al[1]. 2014, n=156	MYBPC3 mutations associated with an older age at diagnosis, first evaluation, and end-stage diagnosis
Page et al[2]. 2012, n=57	MYBPC3 mutations associated with greater disease expression heterogeneity and incomplete, age related and gender specific penetrance
Waldmuller et al[3]. 2011, n=236	MYBPC3 mutations associated with a greater interventricular septum wall thickness
Millat et al[4]. 2010, n=192	MYBPC3 mutations associated with older age
Wang et al[5]. 2008, n=70	MYH7 mutations associated with greater risk of sudden cardiac death among Chinese patients
Song et al[6]. 2005, n=100	MYH7 mutations associated with a younger age at presentation, more frequent syncope and ECG abnormalities.
Van Driest et al[7]. 2004, n=389	MYH7 mutations associated with a younger age and more severe hypertrophy.
Richard et al[8]. 2003, n=197	MYH7 mutations were the most frequent mutations in families with a malignant prognosis.
Charron et al[9]. 1998, n=76	MYBPC3 mutations associated with delayed disease onset and better prognosis
Studies demonstrating no clinical differences between MYBPC3 and MYH7 mutations	

Ellims et al[10]. 2014, n=28	No significant differences in LV dimensions, volumes, maximal wall thickness and LGE burden
Rubinshtein et al[11]. 2010, n=62	No significant differences between the particular HCM gene involved and septal morphology.
Garcia-Castro et al[12], 2009, n=32	No phenotypic differences between patients with various mutations
Van Driest et al[13]. 2004, n=389	Patients with MYBPC3 mutations did not differ from patients with other mutations or genotype negative patients with respect to age at diagnosis, degree of hypertrophy, incidence of myectomy or family history of HCM or sudden death.

Supplementary figure 1 - Unadjusted Kaplan-Meier curve for sudden death up to 5 years of follow-up in patients with MYH7 and MYBPC3 mutations



Supplementary figure 2 - Unadjusted Kaplan-Meier curve for septal reduction up to 5 years of follow-up in patients with MYH7 and MYBPC3 mutations



References

1. Biagini E, Olivotto I, Iascone M, Parodi MI, Girolami F, Frisso G, Autore C, Limongelli G, Cecconi M, Maron BJ, Maron MS, Rosmini S, Formisano F, Musumeci B, Cecchi F, Iacovoni A, Haas TS, Bacchi Reggiani ML, Ferrazzi P, Salvatore F, Spirito P and Rapezzi C. Significance of sarcomere gene mutations analysis in the end-stage phase of hypertrophic cardiomyopathy. *Am J Cardiol*. 2014;114:769-76.
2. Page SP, Kounas S, Syrris P, Christiansen M, Frank-Hansen R, Andersen PS, Elliott PM and McKenna WJ. Cardiac myosin binding protein-C mutations in families with hypertrophic cardiomyopathy: disease expression in relation to age, gender, and long term outcome. *Circ Cardiovasc Genet*. 2012;5:156-66.
3. Waldmuller S, Erdmann J, Binner P, Gelbrich G, Pankuweit S, Geier C, Timmermann B, Haremza J, Perrot A, Scheer S, Wachter R, Schulze-Waltrup N, Dermintzoglou A, Schonberger J, Zeh W, Jurmann B, Brodherr T, Borgel J, Farr M, Milting H, Blankenfeldt W, Reinhardt R, Ozcelik C, Osterziel KJ, Loeffler M, Maisch B, Regitz-Zagrosek V, Schunkert H, Scheffold T and German Competence Network Heart F. Novel correlations between the genotype and the phenotype of hypertrophic and dilated cardiomyopathy: results from the German Competence Network Heart Failure. *Eur J Heart Fail*. 2011;13:1185-92.
4. Millat G, Bouvagnet P, Chevalier P, Dauphin C, Jouk PS, Da Costa A, Prieur F, Bresson JL, Faivre L, Eicher JC, Chassaing N, Crehalet H, Porcher R, Rodriguez-Lafrasse C and Rousson R. Prevalence and spectrum of mutations in a cohort of 192 unrelated patients with hypertrophic cardiomyopathy. *Eur J Med Genet*. 2010;53:261-7.
5. Wang S, Zou Y, Fu C, Xu X, Wang J, Song L, Wang H, Chen J, Wang J, Huan T and Hui R. Worse prognosis with gene mutations of beta-myosin heavy chain than myosin-binding protein C in Chinese patients with hypertrophic cardiomyopathy. *Clin Cardiol*. 2008;31:114-8.
6. Song L, Zou Y, Wang J, Wang Z, Zhen Y, Lou K, Zhang Q, Wang X, Wang H, Li J and Hui R. Mutations profile in Chinese patients with hypertrophic cardiomyopathy. *Clin Chim Acta*. 2005;351:209-16.
7. Van Driest SL, Vasile VC, Ommen SR, Will ML, Tajik AJ, Gersh BJ and Ackerman MJ. Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2004;44:1903-10.
8. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, Benaiche A, Isnard R, Dubourg O, Burban M, Gueffet JP, Millaire A, Desnos M, Schwartz K, Hainque B, Komajda M and Project EHF. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation*. 2003;107:2227-32.
9. Charron P, Dubourg O, Desnos M, Bennaceur M, Carrier L, Camproux AC, Isnard R, Hagege A, Langlard JM, Bonne G, Richard P, Hainque B, Bouhour JB, Schwartz K and Komajda M. Clinical features and prognostic implications of familial hypertrophic cardiomyopathy related to the cardiac myosin-binding protein C gene. *Circulation*. 1998;97:2230-6.
10. Ellims AH, Iles LM, Ling LH, Chong B, Macciocca I, Slavin GS, Hare JL, Kaye DM, Marasco SF, McLean CA, James PA, du Sart D and Taylor AJ. A comprehensive evaluation of myocardial fibrosis in hypertrophic cardiomyopathy with cardiac

magnetic resonance imaging: linking genotype with fibrotic phenotype. *Eur Heart J Cardiovasc Imaging*. 2014;15:1108-16.
11. Rubinshtein R, Glockner JF, Ommen SR, Araoz PA, Ackerman MJ, Sorajja P, Bos JM, Tajik AJ, Valeti US, Nishimura RA and Gersh BJ. Characteristics and clinical significance of late gadolinium enhancement by contrast-enhanced magnetic resonance imaging in patients with hypertrophic cardiomyopathy. *Circ Heart Fail*. 2010;3:51-8.
12. Garcia-Castro M, Coto E, Reguero JR, Berrazueta JR, Alvarez V, Alonso B, Sainz R, Martin M and Moris C. [Mutations in sarcomeric genes MYH7, MYBPC3, TNNT2, TNNI3, and TPM1 in patients with hypertrophic cardiomyopathy]. *Rev Esp Cardiol*. 2009;62:48-56.
13. Van Driest SL, Jaeger MA, Ommen SR, Will ML, Gersh BJ, Tajik AJ and Ackerman MJ. Comprehensive analysis of the beta-myosin heavy chain gene in 389 unrelated patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2004;44:602-10.