

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

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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; $10.4\pm 13.2\%$ versus $8.5\pm 8.5\%$; $P=0.44$) 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 $\approx 35\%$ 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}

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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.

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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 \pm 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.

<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/m²; $P=0.036$). 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*

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*.

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 al³⁶ (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 al²⁵ 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, Olivetto et al³⁹ 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 al⁷ 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 ($\approx 60\%$ 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 al⁴³ 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 al⁴⁴ 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/myofibrillar 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 al⁴⁵ 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 al⁴⁶ 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 al⁴⁷ have shown increased hypertrophy in HCM patients carrying endothelin-1 gene variants. Because HCM is more common among males, Lind et al⁴⁸ 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.

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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; $10.4\pm 13.2\%$ versus $8.5\pm 8.5\%$; $P=0.44$) 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.

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

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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 \geq 30mm, n (%) | 8 (6.2%) | 0 (0%) | 0.32 |

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

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

Continuous variables are presented as mean \pm 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/m ²) | 78.1±17.2 | 80.3±21.7 | 0.56 |
| LVESVi (ml/m ²) | 29±13.7 | 35.7±14.2 | 0.036 |
| LVEF (%) | 64.7±8.9 | 59.6±8.2 | 0.007 |
| Median LVMI (g/m ²) | 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 |

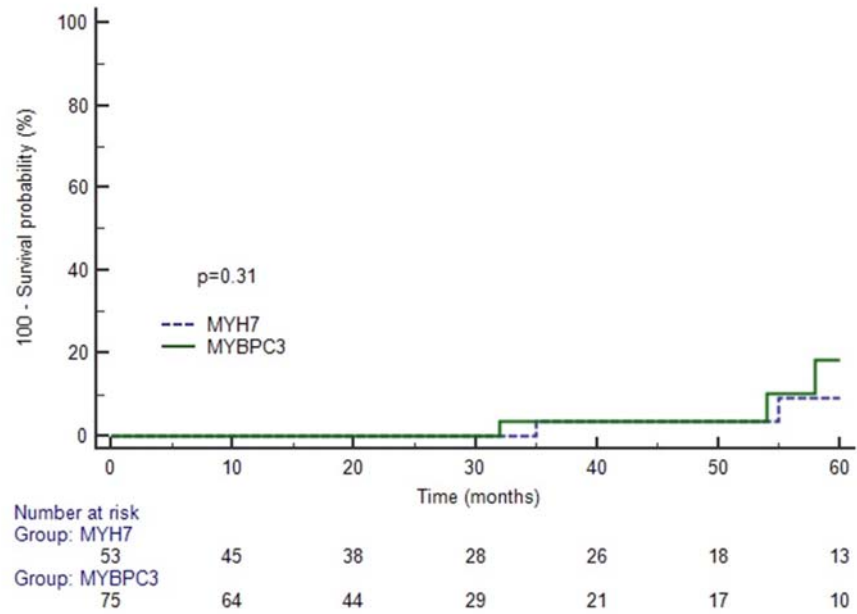
| | | | |
|--|-----------|-----------|------|
| Morphology | | | 0.16 |
| Reversed curvature, n (%) | 68 (53.1) | 11 (35.5) | |
| Neutral septum, n (%) | 33 (25.8) | 8 (25.8) | |
| Sigmoid septum, n (%) | 17 (13.3) | 7 (22.6) | |
| Apical, n (%) | 5 (3.9) | 4 (12.9) | |
| Others, n (%) | 5 (3.9) | 1 (3.2) | |
| <p>Continuous variables normally distributed are presented as mean \pm standard deviation, non-normally distributed as median and interquartile range and categorical variables as percentages.</p> <p>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</p> | | | |

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

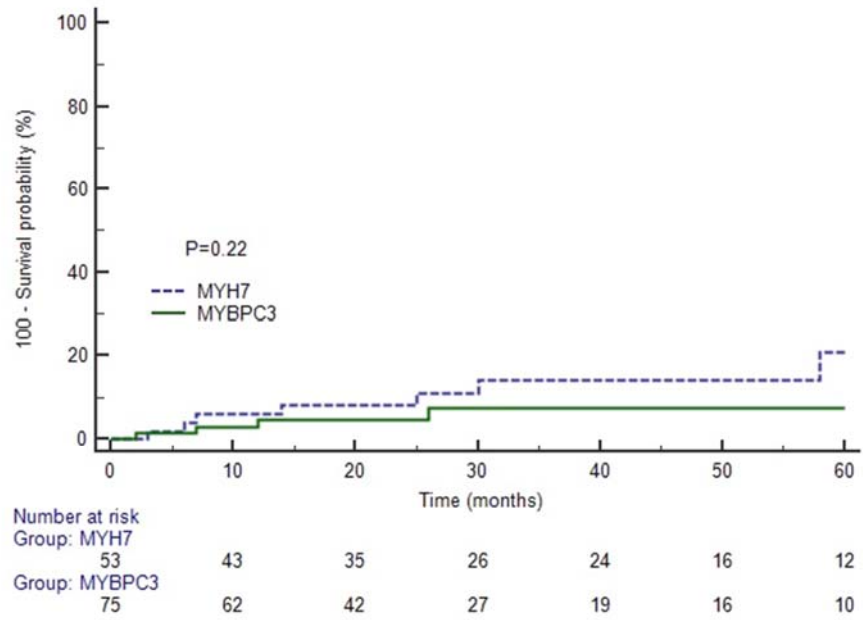
| Studies demonstrating clinical differences between MYBPC3 and MYH7 mutations | |
|---|--|
| Biagini et al ¹ . 2014, n=156 | MYBPC3 mutations associated with an older age at diagnosis, first evaluation, and end-stage diagnosis |
| Page et al ² . 2012, n=57 | MYBPC3 mutations associated with greater 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



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