Worse Prognosis with Gene Mutations of Beta-myosin Heavy Chain than Myosin-Binding Protein C in Chinese Patients with Hypertrophic Cardiomyopathy

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ABSTRACT

Background: No data are available on survival analysis and longitudinal evolution of patients with gene mutations of beta-myosin heavy chain (MYH^7) and myosin binding protein C ($MYBPC^3$) in Chinese.
Hypothesis: To prospectively investigate whether different gene mutations confer distinct prognosis.
Methods: We performed a prospective study in 70 HCM patients and 46 genetically affected family members without HCM-phenotype with direct DNA sequencing of MYH^7 and $MYBPC^3$, clinical assessments, and 5.8 ± 1.8 years follow-up.
Results: After follow-up, more surgical intervention (8/52 versus 0/18, $p<0.001$), higher sudden death risk (7/52 versus 0/18, $p<0.001$) and shorter life span were found in patients with MYH^7 mutations than in patients with $MYBPC^3$ mutations (45.1 ± 14.0 versus 73.5 ± 7.5 years, $p = 0.03$). Seven of the 27 mutation carriers of MYH^7 had clinical presentations of HCM, but no carriers of $MYBPC^3$ mutations developed to HCM during follow-up. Maximal wall thickness was thicker in the patients carrying mutations in the global region of MYH^7 than in those carrying mutations in the rod region of MYH^7 (21.5 ± 6.6 versus 15 ± 6.1 mm, $p<0.05$) at baseline. More sudden death (7/41 versus 0/11) and left ventricular dysfunction (NYHA Class III $\sim$ IV, 17/32 versus 1/10) were identified in patients with mutations in the global region of MYH^7 than in patients with other mutations.
Conclusions: MYH^7 mutations, especially in the global region, cause malignant clinical phenotypes

Key words: genotype, prognosis, hypertrophic cardiomyopathy

Introduction

On the basis of genotype–phenotype correlations studies in large hypertrophic cardiomyopathy (HCM) pedigrees, genetic defects causing HCM could represent the primary determinant and stratifying marker of prognosis, sudden cardiac death, and heart failure. Mutations in different genes or specific regions of one gene may have different clinical features.[1,2] Certain mutations of *MYH*^{7} have been associated with a significantly shorter life span in patients with HCM,[3] whereas, mutations in *MYBPC*^{3} have been associated with late onset of hypertrophy and a relatively benign prognosis.[4] Recently, large studies on HCM patients do not support a genotype–phenotype correlation.[5]

Published clinical data assembled over clinical implications of HCM and the treatment strategies were mostly from a few selected tertiary centers in North America or Europe. Clinically stable and asymptomatic elderly patients, and patients other than from North America or Europe were under-represented.

At present, it is not clear whether there is a particular prognosis for the adults harboring different mutant HCM genes but having neither left ventricular hypertrophy nor other clinical phenotypes of HCM, or for HCM patients carrying different mutations.

In order to prospectively investigate whether different gene mutations confer a distinct prognosis, we followed up on a total of 70 HCM patients carrying either *MYH*^{7} mutations or *MYBPC*^{3} mutations, and 46 subjects carrying the mutation without clinical phenotype.

Methods

Patient Selection

The recruitment of HCM patients has been described previously.[6,7] The HCM patients were consecutively recruited in Fuwai Hospital and Cardiovascular Institute,

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Chinese Academy of Medical Sciences. Clinical evaluation included medical history, physical examination, 12-lead electrocardiography, M-mode, two-dimensional and Doppler echocardiography. A blood sample for DNA analysis was taken from patients and family members of patients after informed written consent. Clinical records and family history were obtained to determine the disease-related deaths and the age at death for all affected individuals in each family.

A total of 70 HCM patients and 46 genetically affected family members without HCM-phenotype, but harboring mutations in *MYH*[7] or *MYBPC*[3], were followed up on at Cardiovascular Institute and Fuwai Hospital, Chinese Academy of Medical Sciences from July 1997 to January 2006. The initial clinical evaluation was defined as the time when echocardiography and physical examination were first assessed at Fuwai Hospital from July 1997 to October 2001.

The mean (± SD) duration of follow-up was defined as the duration from initial clinical visit (echocardiography and physical examination) to the most recent evaluation, or death.

The clinical parameters recorded during follow-up included cardiac symptoms, survival status (survival or death), New York Heart Association (NYHA) Functional Classification, electrocardiogram, and echocardiography.

The study protocol was reviewed and approved by the Review Board of Beijing Municipal Commission for Science and Technology and Center for Molecular Cardiology, to conform to the principles outlined in the Declaration of Helsinki for human study (1997). Informed consents were obtained from all subjects for agreeing to use their medical information for research purposes.

Mutation Detection and Localization

Methods for screening of the coding sequences of *MYH*[7] and *MYBPC*[3] were as described previously.[6]

The mutations in *MYH*[7] were located in two regions according to the structural-functional domains, the global region, containing the site for actin binding, active site, essential light chain binding site, and the rod region mutations referring to the mutation located in the region of head-rod junction and the rod of *MYH*[7].[8]

Echocardiography and Definition

Echocardiographic studies were described previously.[6,7] The diagnosis of HCM was based on echocardiographic criteria previously described,[7] that is, the maximal thickness of the left ventricular wall was >13 mm, measured in diastole, in the region of greatest hypertrophy in absence of any other causes of ventricular hypertrophy. Left ventricular outflow obstruction was defined as a peak instantaneous gradient 30 mm Hg or more under resting conditions.[9]

Sudden death was defined as sudden and unexpected death due to HCM occurring within 1 hour from the onset of symptoms in patients previously experiencing a relatively stable clinical course.[9] Death was also classified as sudden if it occurred unexpectedly but was unwitnessed, such as death occurring in bed during the night.

Death related to heart failure was defined as that occurring in the context of cardiac decompensation and a progressive course with limiting symptoms, particularly when it was complicated by pulmonary edema or required hospitalization, or both.[9] One patient received a heart transplant due to advanced refractory heart failure and was classified under death due to HCM-related heart failure.[9]

Stroke-related deaths were judged as a consequence of embolic events related to HCM in the presence of atrial fibrillation.[9]

The major intervention for patients was defined as surgical septal myectomy, alcohol septal ablation, and dual-mode, dual-pacing, and dual-sensing (DDD) pacemaker.

The new patient was diagnosed if they met the echocardiographic criteria previously described[7] or an abnormal electrocardiography[10] occurred in patients carrying *MYH*[7] or *MYBPC*[3] mutations.

Statistic Analysis

Data are expressed as means ± SD. An unpaired Student *t* test was employed for comparison of continuous data between different genes and different regions of *MYH*[7]. A paired sample t-test was used to test the differences of continuous data between follow-up and baseline. The chi-square test was utilized to test noncontinuous variables expressed as proportions. Survival curves were constructed according to the Kaplan–Meier method. Calculations were performed with SPSS 13.0 software (SPSS Inc., Chicago, Ill., USA).

Results

The Characteristics of Patients with Different Gene Mutations

The onset age was 16 years younger in patients with *MYH*[7] mutations than in those with *MYBPC*[3] mutations (34.6 ± 14.0 versus 50.0 ± 15.0 years, $p<0.001$). The ratio of family history of sudden death was also higher in patients with *MYH*[7] mutations than in those with *MYBPC*[3] mutations (27/52 versus 1/18, $p<0.001$). Higher proportions of atrial fibrillation (12/52 versus 1/18, $p = 0.09$) and of resting left ventricular outflow obstruction (21/52 versus 2/18, $p = 0.07$) were identified in patients with *MYH*[7] mutations, than in patients with *MYBPC*[3] mutations, but did not reach statistical significance. No differences were found in the other clinical manifestations, electrocardiography, and echocardiography between the two groups (Table 1).

Different Gene Mutations Led to Distinct Prognosis

The mean duration of follow-up was 5.9 ± 1.8 years in patients with *MYH*[7] mutations and 5.7 ± 1.7 years in patients with *MYBPC*[3] mutations ($p>0.05$). During follow-up, patients with *MYH*[7] mutations had more surgical intervention (8/52 versus 0/18, $p<0.001$) than did those with *MYBPC*[3] mutations, including surgical septal myectomy

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TABLE 1: Characteristics of patients with mutations in *MYH*[7] and *MYBPC*[3]

	MYH[7] (n = 52)		*MYBPC*[3] (n = 18)	
Phenotype	At presentation	At recent evaluation	At presentation	At recent evaluation
Male, n (%)	27(51.9)		13(72.2)	
Family history of sudden death, n (%)	27(51.9)[a]		1(5.6)	
Age (yrs)	34.6 ± 14.0[b]	39.9 ± 13.7	50.0 ± 15.0	54.7 ± 13.6
NYHA Class, n (%)				
Class I ~ II	38(73.1)	31(59.6)	15(83.3)	12(66.7)
Class III ~ IV	14(26.9)	21(40.4)	3(16.7)	6(33.3)
Atrium fibrillation, n (%)	12(23.1)[a]	16(30.8)	1(5.6)	4(22.2)
Echocardiography				
LVEDD (mm)	44.2 ± 5.9	45.6 ± 6.7	46.6 ± 3.6	46.9 ± 4.3
MLVWT (mm)	20.5 ± 5.7	19.2 ± 5.3	19.9 ± 6.5	19.4 ± 6.8
PWT (mm)	10.3 ± 2.1	9.8 ± 1.7	10.6 ± 2.6	10.2 ± 2.4
LAD (mm)	41.7 ± 6.7	42.6 ± 6.9	44.6 ± 6.8	44.8 ± 7.1
LVOG>30 mmHg	21(40.4%)	18(34.6%)	2(11.1%)	2(11.1%)

[a] $p<0.05$ between the patients with mutations in two genes at presentation. [b] $p<0.01$ between the patients with mutations in two genes at presentation.
Abbreviations: LAD = Left atrial diameter; LVEDD = Left ventricular end-diastolic dimension; LVOT = Resting left ventricular outflow tract; MLVWT = Maximal LV wall thickness; NYHA = New York Heart Association; PWT = Left ventricular posterior wall thickness.

(3/8), alcohol septal ablation (1/8), and DDD pacemaker (4/8). Fourteen patients died, 10 of them had *MYH*[7] mutations and 4 had *MYBPC*[3] mutations. The sudden death ratio was higher (7/52 versus 0/18, $p<0.001$) and the age at death was younger (45.1 ± 14.0 versus 73.5 ± 7.5 years, $p = 0.03$) in patients with *MYH*[7] mutations than in those with *MYBPC*[3] mutations (Table 2, Figure 1). The patients with *MYBPC*[3] mutations had a normal life span (73.5 ± 7.5 years, range, 67.4–84.5 years). Sudden death occurred only in the patients carrying mutations of R663C (1/7), A26V (3/7), Q734P (2/7) and R719Q (1/7) in *MYH*[7].

MYH[7] Mutations were Much More Frequently Evolved to HCM than were *MYBPC*[3] Mutations in Clinically Unaffected Subjects at the Beginning

At baseline, 46 subjects carry the mutations either in *MYH*[7] (27) or in *MYBPC*[3] (19) with no clinical, electrocardiographic, and echocardiographic HCM manifestations. During follow-up, 9 subjects with *MYH*[7] mutations (4 with A26V, 3 with R143Q, and 2 with R663H) developed symptoms including dyspnea (3/9), angina (1/9), palpitation (4/9), or syncope (1/9), and abnormal electrocardiography (5/9) or thicker interventricular septum (7/9) in echocardiography. All 9 new patients carry *MYH*[7] mutations, and their mean onset ages were 37.3 ± 5.6 years. The *MYBPC*[3] mutation carriers had neither clinical symptoms nor presentations of HCM during the entire period of follow-up. The mean age of those *MYBPC*[3] mutation carriers were 46.8 ± 9.7 years old at the last time of evaluation.

Mutations in the Global Region Led to More Malignant Clinical Manifestations than Mutation in the Rod Region in *MYH*[7]

According to the mutation location of the substituted amino acid, patients with *MYH*[7] mutations were subclassed into mutations in the global region (41/52) and in the rod region (11/52); with charge change (24/52), and without charge change (28/52). At baseline, the maximal wall thickness was thicker in the patients with mutations in the global region than in those with mutations in the rod region (21.5 ± 6.6 versus 15 ± 6.1 mm, $p<0.05$). During follow-up, a total of 10 patients died, 3 of heart failure and 7 due to sudden death. All 7 sudden death patients carried *MYH*[7] mutations in the global region; only one patient who died of heart failure carried the mutation in the rod region (Figure 1).

At the final evaluation, 7 out of 29 patients with mutations in the global region had NYHA Class I~ II at baseline, which developed to Class III ~ IV during follow-up, whereas no patient with mutation in the rod region had cardiac function

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TABLE 2: The 6-year outcome of patients with different gene mutations

Characteristics	HCM-causing gene		p-Value
	MYH^7 (n = 52)	*MYBPC*^{3} (n = 18)	(*MYH*^{7} versus *MYBPC*^{3})
Duration of follow-up (yrs)	5.9 ± 1.8	5.7 ± 1.7	ns
Major intervention for patients,[a] n	8	0	<0.001
Death related to HCM, n, (%/1,000 person-year, 95% CI)	10 (32.1, 12.5–51.5)	4 (35.2, 13.9–68.9)	ns
			ns
Sudden death	7	0	<0.001
Stroke	0	1	–
Heart failure	3	3	–
Age at death (yrs)	45.1 ± 14.0	73.5 ± 7.5	0.03
NYHA Class III ~ IV	6	1	ns

[a] The major intervention included surgical septal myectomy, Alcohol septal ablation and DDD pacemaker. *Abbreviations:* NYHA = New York Heart Association.

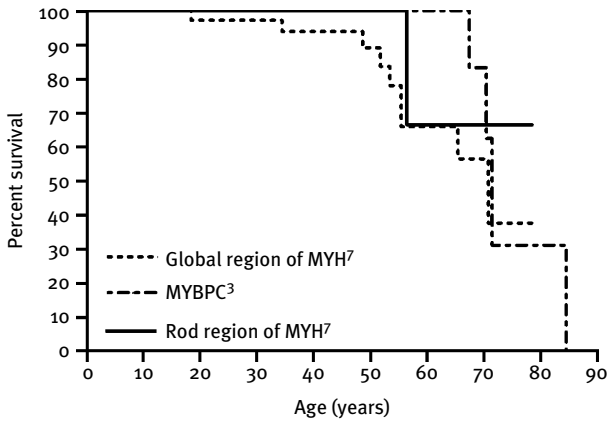

Figure 1: Kaplan–Meier curves for the survival of patients with mutations in the globe and rod region of *MYH*^{7} and *MYBPC*^{3}.

NYHA Class III ~ IV ($p<0.01$ between the two groups) during follow-up.

The charges of the mutated amino acids had no significant influence on clinical presentation and prognosis at baseline and after a 6-year follow-up.

Discussion

In this prospective study, we found that patients with mutations in different genes have distinct clinical presentation and prognosis. The patients with *MYH*^{7} mutations had malignant clinical manifestation, shorter life span, and a higher risk of sudden death than did the patients with *MYBPC*^{3}. In *MYH*^{7} mutations, patients with mutations in the global region led to thicker maximal wall thickness at baseline, more sudden death, and higher left ventricular dysfunction during follow-up. In our prospective cohort study, we found that *MYH*^{7} mutations, especially in the global region, cause malignant clinical phenotypes.

We investigated the co-segregation of the mutations in the genotype-positive, genotype-negative, and control cohorts, and found that the mutations are co-segregated with HCM within a family, and not for reference alleles during a 6-year follow-up. This further confirms that the mutations we reported here were HCM-causing mutations.

Patients with heart failure also are at high risk of sudden death.[11] End-stage HCM patients' cases are usually complicated with heart failure. Therefore, differentiating sudden death with death due to heart failure is difficult. In our study, sudden death was diagnosed only when death due to HCM occurred within 1 h from the onset of symptoms in patients previously experiencing a relatively stable clinical course. When death occurred in the context of cardiac decompensation and a progressive course with limiting symptoms, death related to heart failure was diagnosed, particularly when it was complicated by pulmonary edema, or required hospitalization, or both.[9]

Disagreement exists in the definition of "malignant" mutations. Some hot "malignant" mutations have not been correlated with the degree of cardiac hypertrophy, family history of HCM, or sudden death in a large HCM cohort.[12] The A26V mutation was found to have a mild phenotype in another Chinese family with HCM.[13] Many speculations have been proposed to explain the intrafamily variation. First, "malignant" mutations may combine with other multiple mutations unidentified in the sarcomeric

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contractile proteins, other nonsarcomeric proteins, the mitochondrial genome, or even with unknown HCM-causing genes to cause the most severe phenotype.[5] In our recent study, we screened full encoding sequences of *MYH*[7], *MYBPC*[3], *TNNT*[3], and *TNNI*[2], and even though no other mutations were found in the subjects, we still cannot fully rule out that unidentified mutations in noncoding regions contribute to the phenotype of HCM. Second, the expression of phenotype may also be influenced by other genetic factors, so-called modifier genes.[2] Third, geographical and racial background may influence the expression of phenotype in these patients.

It has been reported that *MYH*[7] mutations cause high penetrance and early onset of HCM, the penetrance of *MYBPC*[3] mutation are age-dependent in most previous studies.[3,4] Yet, from our study, the penetrance of *MYH*[7] mutation is also age-dependent. No change was found in the subjects carrying *MYBPC*[3] mutations during a 6-year follow-up. Longer time is needed to evaluate the penetrance of *MYBPC*[3].

There are different results on whether the charge changes of the mutations or critical regions of mutations are more important on prognosis of HCM patients.[3,8,14] We found that the change in overall charge of the substituted amino acid is irrelevant to sudden death and survival. Our results support that the functional region, not the charge change of the mutated amino acid in *MYH*[7], predicts prognosis.

Limitation

Our study still carries some limitations, the sporadic patients and probands were recruited from our tertiary referral center, which had some selection bias relative to community-based patients. Second, our study sample size was relatively small, a larger sample size of prospective cohort study is needed to confirm our conclusion.

Conclusion

In conclusion, this prospective study implies that patients with mutations in *MYH*[7], particularly in the global region, had malignant clinical manifestation, shorter longevity, and high risk of sudden death, not *MYBPC*[3]. Our results provide potential guidance on risk stratification of patients with HCM, and subjects carrying gene mutations with no clinical phenotypes.

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