

# Mutation of V896M in cardiac myosin binding protein-c gene in two Chinese families with hypertrophic cardiomyopathy

AI-LING WANG<sup>1</sup>, DE-HUA KONG<sup>1</sup>, DUO-XUE CHEN<sup>1</sup>, JUN WAN<sup>1</sup> and YUAN-XUN YU<sup>2</sup>

<sup>1</sup>Department of Cardiology, The First Affiliated Hospital of Anhui Medical University, Hefei 230022;

<sup>2</sup>The Center of Medical Genetics of Anhui Province, Hefei 230031, P.R. China

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**Abstract.** To investigate the genotype-phenotype correlation in Chinese familial and sporadic hypertrophic cardiomyopathy, specific exons of the myosin binding protein-c gene (*MYBPC3*) were screened in six families with hypertrophic cardiomyopathy (HCM; FHCM) and in 20 patients with sporadic HCM (SHCM) from the Anhui Province region of China. The V896M mutation was detected for the first time in China in two families with FHCM. The mutation was not found in 100 healthy control subjects. No mutations of *MYBPC3* were detected in any of the SHCM patients. In contrast to previous reports, the V896M mutation may be a disease-causing mutation in China, and exon 27 of *MYBPC3* may be a mutational hotspot in FHCM patients. However, mutations of *MYBPC3* were not prevalent among SHCM patients.

## Introduction

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiac disease, and is characterized by a very heterogeneous morphologic expression and clinical course (1,2). With an estimated prevalence in the general population of approximately 1:500, there are potentially over one million patients with HCM in China alone (3). Genetic screening is a necessary tool for a definite diagnosis of HCM, particularly in subjects with atypical or borderline phenotypic expression (4). Several sarcomeric and non-sarcomeric protein genes have been associated with the disease, and novel culprit genes are being described every year. Thus, comprehensive molecular screening for HCM represents a technical and economic challenge and is hardly feasible in China. The two most common sarcomeric genes involved in HCM are  $\alpha$ -myosin heavy chain (*MYH7*) and cardiac myosin binding protein-c

(*MYBPC3*). These genes account for a proportion of disease-associated mutations that was consistently high in Europe (5), despite wide fluctuations attributable to the different genetic backgrounds of the study subjects. Based on the screening of *MYH7* in six families one year ago, we determined that the comprehensive screening of *MYBPC3* may provide a favorable yield of genotyped index cases. The present study was undertaken in the same six unrelated Chinese families with HCM (FHCM) and in 20 patients with sporadic HCM (SHCM) at the Department of Cardiology of The First Affiliated Hospital of Anhui Medical University.

## Materials and methods

**Clinical materials.** A total of 20 patients in six unrelated families (8 males and 12 females; age range 17-70 years) and 20 SHCM patients were identified through the patient register of the Department of Cardiology of our hospital and evaluated. The main clinicopathologic findings of these patients are summarized in Table I. The diagnosis of HCM was based on the presence of a maximal left ventricular wall thickness of at least 13 mm, determined by two-dimensional echocardiography. The absence of other causes of ventricular hypertrophy, such as hypertension, aortic stenosis, physical exercise or the use of some drugs, was confirmed in each patient. Electrocardiographic and echocardiographic examinations were also performed in the six families. All individuals gave their informed consent for participation. The study was approved by the Ethical Committee of the hospital. To confirm the absence of any new putative mutation in the *MYBPC3* gene among the healthy individuals, a total of 100 controls were genotyped. The controls did not have a history of cardiovascular disease, including HCM.

**Genetic studies.** *MYBPC3* sequencing of genomic DNA was prepared from peripheral blood leukocytes using a salting-out procedure. Exons 13, 15-16, 26 and 27 of the *MYBPC3* gene, as well as the corresponding exon-intron boundaries (gi:89161188) (Table II), were sequenced in all probands of the six families and 20 SHCM patients. PCR reactions were performed in a GeneAmp PCR system (Hema-240; PCR Zhuhai Biotechnology Group, P.R. China). Each reaction included 1,000 ng of genomic DNA, 7.5 pmol of each primer, 500  $\mu$ mol of dNTP, 2.5  $\mu$ l 10X buffer and 2.0 units *Taq* DNA

**Correspondence to:** Dr Ailing Wang, Department of Cardiology, The First Affiliated Hospital of Anhui Medical University, Hefei 230022, P.R. China  
E-mail: wal@ah.edu.cn

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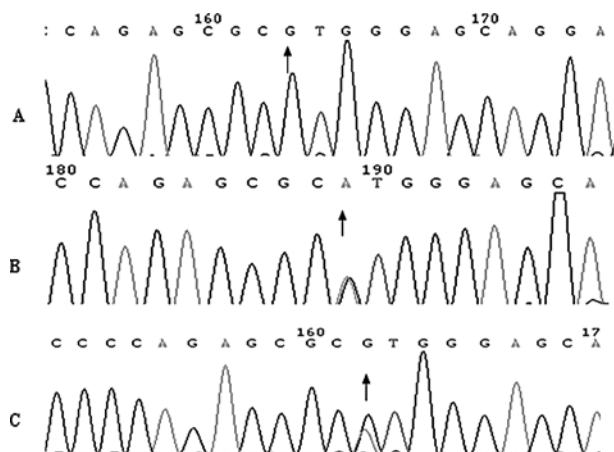
Table I. Main characteristics of the five patients with HCM in the two families showing the V896M mutation.

Subjects	Gender/age	Angina	Syncope	NYHA	ECG			UCG (mm)		
					LVH/ST	Q/BBB	IVS/PW	LVDD	LAD	EF (%)
DI:1	F/63	+	-	III	+ -	+	15/10	58	44	48
DII:1	M/40	-	-	II	- -	-	14/11	52	56	68
EI:2	M/69	-	-	IV	+ +	-	19/11	65	58	45
EII:1	F/46	-	-	III	+ -	+	14/12	57	45	50
EII:3	F/43	-	-	II	- -	-	15/11	53	57	69

ECG, electrocardiography; M, male; F, female; LVH, left ventricular hypertrophy; IVS, interventricular septal thickness; PW, posterior left ventricular wall; LVDD, LV end-diastolic dimension; EF, ejection fraction; LAD, left atrial diameter.

Table II. Primers and PCR conditions for analysis of the *MYBPC3* gene.

Exon	Primers		Annealing (°C)
	Forward	Reverse	
Exon 13	5'tccccagcccctcttca3'	5'gccggactccgctcttt3'	62
Exon 15 + 16	5'ctctcctttgtctcgggct3'	5'gggtgagcatgagggttggc3'	62
Exon 26	5'aacagatccgaggggaaggtgg3'	5'ttttaactggggaggggggc3'	59.5
Exon 27	5'ggaagtgcccccctatgt3'	5'tcgactgctcaagaag3'	62

Figure 1. Sequencing analysis of exon 27 of the *MYBPC3* gene. (A) wild-type; (B) family D; (C) family E. Partial sequence of exon 27 of *MYBPC3* showing the heterozygous GTG-(G/A) TG substitution at position nt16750 (indicated by the arrow).

polymerase (Sangon Biotechnology, P.R. China). The PCR conditions for the cardiac *MYBPC3* gene were denaturing at 94°C for 4 min, followed by 32 cycles of denaturing at 94°C for 40 sec, annealing at 59.5-62°C for 40 sec and extension at 72°C for 90 sec, with a final extension at 72°C for 10 min. Genomic DNA was PCR-amplified with the primer pairs and annealing temperatures summarized in Table II. Each PCR product was analyzed on an ABI PRISM 3730XL automated sequencer (Perkin-Elmer Applied Biosystems, USA)

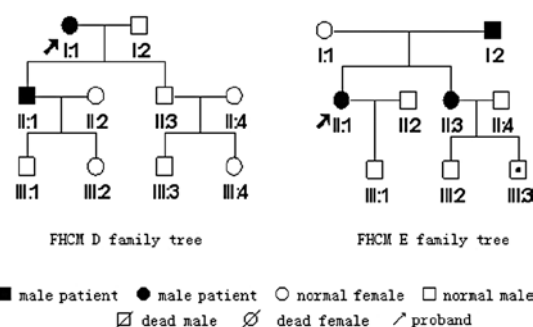


Figure 2. Pedigrees of two of the six families, showing the V896M mutation.

according to dideoxy chain termination reaction sequencing reactions.

## Results

**Identification of the V896M-MYBPC3 missense mutation.** A 16750 G→G/A mutation at exon 27 of the *MYBPC3* gene was detected in two probands (families D and E) among the six families, resulting in the replacement of a valine by methionine residue at amino acid 896 (gi:148596956) (Fig. 1). None of the 100 control subjects had the mutation. We then screened exon 27 of the *MYBPC3* gene in other members of the same two families. The results showed that the mutation co-segregated with the disease (Fig. 2, Table III). Mutations of *MYBPC3* were not detected in any of the SHCM patients.

Table III. Variants in the *MYBPC3* and *MYH7* genes in six pedigrees with HCM and 20 SHCM patients.

Family	Nucleotide change	Predicted change in protein	Previous publication	No. of probands(n=5)/relatives with variant	No. of controls (n=80) with variant
A	<i>MYH7</i> Exon 18 <sup>a</sup> (nt12601: G→A/G)	Arg663His	Yes	1/3	0
B	<i>MYH7</i> Intron 18 (nt9088: deletion A)	No	No	1/2	3
	<i>MYH7</i> Exon 20 <sup>a</sup> (nt13619: C→C/G)	Arg723Gly	Yes	1/5	0
C	<i>MYH7</i> Intron 18 (nt9085: G→A/G)	No	No	1/2	1
	<i>MYH7</i> Exon 20 <sup>a</sup> (nt13659: T→C/T)	Ile736Thr	Yes	1/3	0
D	<i>MYBPC3</i> Intron13 (nt6628: G→G/A)	No	No	1/2	2
	<i>MYBPC3</i> Exon 27 (nt16750: G→G/A)	Val896Met	Yes	1/2	0
	<i>MYBPC3</i> Intron 26 (nt16647: C→C/T)	No	No	1/2	1
E	<i>MYBPC3</i> Exon 27 (nt16750: G→G/A)	Val896Met	Yes	1/4	0
	<i>MYBPC3</i> Intron 27 (nt16813: C→C/T)	No	No	1/4	1
	<i>MYBPC3</i> Intron 15 (nt9444: G→G/T)	No	No	1/3	4
S1	<i>MYH7</i> Exon 20 <sup>a</sup> (nt13659: T→C/T)	Ile736Thr	Yes	1/1	0

S1, first sporadic patient; <sup>a</sup>previously reported.

**Genetic and clinical features.** Two pedigrees (families D and E) were selected for genetic study. All affected members presented typical clinical features of the disorder as assessed by physical examination, electrocardiography, two-dimensional echocardiography and Doppler echocardiography. The disease was inherited as an autosomal dominant trait in all cases, as documented by the patient history or clinical evaluation of the relatives. All families were unrelated and of Chinese descent.

In family D, two of the ten members were confirmed to carry the mutated gene. The proband (DI:1) was a 63-year-old man who presented with occasional symptoms of angina. Electrocardiography (ECG) showed left ventricular hypertrophy with left bundle branch block. Echocardiography revealed a ventricular septum thickness of 15 mm and an ejection fraction (EF) of 48%. His son (DII:1) was 40 years of age, and was diagnosed with HCM 2 years prior due to chest stress. Echocardiography showed an interventricular septum thickness of 14 mm and posterior wall thickness of 11 mm.

The dimension of the left atrium was 56 mm while that of the left ventricular diastolic was 52 mm. The EF of 68%.

In family E, the V896M mutation of the *MYBPC3* gene was detected in four cases. Three members were diagnosed with HCM. The proband (EII:1), a 46-year-old woman, was diagnosed with HCM due to exertional chest stress 4 years prior. The father of proband was a 69-year-old man with a New York Heart Association functional class (NYHA FC) of IV who presented with frequent symptoms of chest stress. Echocardiography showed an interventricular septum thickness of 19 mm and posterior wall thickness of 11 mm. The dimension of the left atrium was 58 mm, while that of the left ventricular diastolic was 65 mm. The EF was 45%. The sister of the proband (EII:3) was 43 years of age, and was diagnosed with HCM one year prior during a health examination. Finally, EIII:3 was a 15-year-old boy determined to carry the V896M mutation, and was confirmed to be healthy through electrocardiographic and echocardiographic examination.

None of the patients were found to have left ventricular outflow obstruction, as determined by echocardiography, and none of all patients succumbed suddenly to the disease among the two families.

## Discussion

HCM is generally considered to be a strictly autosomal dominant inherited disease, and manifests as left ventricular or interventricular septal asymmetrical hypertrophy. It exhibits variability in its phenotypes, with clinical manifestations including dizziness, dyspnoea, syncope, angina, serious heart failure, and even sudden death (1). However, symptoms may also be absent, non-specific or late in onset. HCM is largely due to mutations in the genes encoding proteins of the sarcomere. Therefore, it is also called 'sarcomere disease' (6).

The sarcomere complex is composed of thick and thin filaments, and plays a role in the contraction, construction and regulation of human myocardium filaments. MyBPC is a sarcomeric protein associated with the thick filament of striated muscle. It is located in the cross-bridge-containing A-band of the sarcomere in a structurally regular pattern of seven to nine (7). The core structure of MyBPC comprises seven class I immunoglobulin domains and three fibronectin type III (FnIII) domains, numbered from the N-terminus as domains 1-10 (C1-C10) in skeletal muscle. MyBPC has been assigned roles in both the structural assembly and stability of the sarcomere, as well as in the modulation of contraction.

The V896M mutation is located on the field of C7, while domains C7-C10 belong to the C-terminal part of the protein and have a high affinity for the light meromyosin (LMM) portion of myosin and for titin, the structural 'ruler' of the sarcomere. The C-terminal domain, C10, binds to four molecules of LMM via positively-charged amino acids present on its surface (8). However, even though this domain is essential, it is not sufficient for maximal binding to myosin. The three adjacent domains (C7-C9) maximise the affinity of this binding and ensure correct incorporation into the A-band (9).

The V896M mutation was first reported in two South African subpopulations by Moolman-Smook *et al* (10). A subsequent study by Richard *et al* (11) showed that the presence of a heterozygous *MYBPC3* variant (V896M) contributed to the phenotype severity, and was associated with early hypertrophy when present together with a heterozygous A355T *MYH7* mutation. The authors suggested that the V896M variant acts as a modifier polymorphism on the expression of the phenotype.

While a previous study identified the V896M variant in 5/111 control individuals in (12), it was not found among the healthy controls in our study, and the mutation and the disease showed co-separation. It may therefore be a disease-causing mutation in China.

In the two families with the V896M mutation discussed here, the V896M variant of *MYBPC3* was detected in six cases. Five of six family members were diagnosed as having HCM. Only one was confirmed to be a healthy carrier through electrocardiographic and echocardiographic examinations. However, this one (EIII:3) is a 15-year-old boy, and studies have shown that patients with *MYBPC3* mutations usually have a delayed age of onset (13). Long-term follow-up is therefore essential in this patient.

The penetrance in our study was calculated to be 83% (5/6 carriers). There were no obstructions in the left ventricular outflow tract in any of the patients, as determined by echocardiographic examination, and none of the patients in the two pedigrees succumbed suddenly to the disease. The carriers who were over 38 years of age had a normal left ventricular dimension and EF, but presented ventricular wall hypertrophy. Most of the 60-year-old carriers presented heart failure and a gradual decrease in the EF. Taken together, the results suggest that the phenotype becomes more malignant with increasing age. Thus, in the present study the V896M mutation was found to exhibit similar features to those reported by Richard *et al* (13), including the delayed onset of clinical symptoms, slow development and a benign phenotype.

We previously reported three mutations (Family A, Arg663His of exon 18; Family B, Arg 723Gly of exon 20; Family C, Ile736Thr of exon 20; and Ile736Thr of exon 20 in 1 of 20 SHCM patients) of the *MYH7* gene in the same six families and among 20 SHCM patients (Table III) (14). Here, we report a mutation in the *MYBPC3* gene in two of the same six families with HCM. In five of the six families, mutations in the *MYBPC3* and *MYH7* genes were detected. However, only 1 SHCM patient was found to have a Ile736Thr mutation of exon 20 in the *MYH7* gene. This indicates that mutations of the *MYBPC3* and *MYH7* genes frequently occur among FHCM patients, but not among SHCM patients, in China.

In conclusion, we showed a missense mutation, V896M, in two Chinese families with a benign phenotype of HCM with mild clinical symptoms. *MYBPC3* as well as *MYH7* may be the dominant disease-causing genes in Chinese familial HCM patients, while the mutation rate of the *MYBPC3* and *MYH7* genes is significantly lower among sporadic HCM patients. Exon 27 of the *MYBPC3* gene may be a mutational hotspot among Chinese FHCM patients.

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