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Original article

Characteristics of the beta myosin heavy chain gene Ala26Val mutation in a Chinese family with hypertrophic cardiomyopathy

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Abstract

Background: Genotype–phenotype studies have suggested that some mutations of genes encoding various components of the cardiac sarcomere cause hypertrophic cardiomyopathy (HCM) and are associated with the prognosis of patients with HCM. The aims of this study were to investigate the gene mutations of exons in the cardiac beta myosin heavy chain (MYH7) gene, the troponin T (TNNT2) gene, and the brain natriuretic peptide (BNP) gene, as well as to assess the effect of these mutations on the clinical features of Chinese patients with HCM.
Methods: Five unrelated Chinese families with HCM were studied. Exons 3 and 18 in the MYH7 gene, exon 9 in the TNNT2 gene, and all three exons in the BNP gene were screened with the polymerase chain reaction (PCR) for genomic DNA amplification. Further study included purification of PCR products and direct sequencing of PCR fragments by fluorescent end labeling.
Results: A C-to-T transition in codon 26 of exon 3 in the MYH7 gene was found in one family (including four patients and five carriers), resulting in an amino acid substitution of valine (Val) for alanine (Ala). The Ala26Val mutation was of incomplete dominance (penetrance 44%). This mutation was not seen in the other four families or in the control group. Moreover, the association between the gene mutations of exon 18 in MYH7, of exon 9 of TNNT2, and of all three exons in BNP and HCM was not found in the populations we studied.
Conclusions: The missense mutation Ala26Val found in this one Chinese family was associated with a mild phenotype of HCM. The genetic and phenotypic heterogeneity of HCM exists in the Chinese population. It suggests that genetic and environmental factors may be involved in the pathogenesis of HCM.

Keywords: Cardiomyopathy; Hypertrophy; Mutation; Genetics

1. Introduction

Hypertrophic cardiomyopathy (HCM) is a cardiac disorder characterized by left ventricular hypertrophy (LVH) with predominant involvement of the interventricular septum in the absence of other causes of hypertrophy [1]. It is also characterized by an autosomal-dominant mode of inheritance. To date, nine genes encoding various components of the cardiac sarcomere have been implicated in HCM: cardiac beta myosin heavy chain (MYH7), troponin T (TNNT2), alpha tropomyosin (TPM1), myosin binding protein C3 (MYBPC3), cardiac ventricular myosin light chain (MYL2), cardiac myosin alkali light chain (MYL3), troponin I (TNNI3), alpha cardiac actin (ACTC), and titin (TTN). Numerous mutations have been described in these genes [2,3].

Recent genotype–phenotype studies have suggested that some mutations may be associated with a high incidence of disease-related mortality, whereas others appear to have a more "benign" course [4,5]. Mutations in MYH7 are the most commonly described defects in

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HCM and the most frequent causes of familial HCM [6,7]. Mutations in TNNT2, particularly R92W (exon 9), have been associated with a high incidence of sudden cardiac death (SCD) in spite of minimal hypertrophy [8]. Recently, the missense mutations in exons 3 and 18 in the MYH7 gene were identified in Chinese patients with HCM [9,10]. These gene mutations may be distributed differently in different populations and may have variable phenotypic expression among affected individuals in Chinese populations with HCM. Plasma levels of brain natriuretic peptide (BNP) in HCM patients without heart failure were higher than those in healthy control subjects [11]. The high excretion and expression of BNP may be secondary to cardiac overload. It is unclear whether the gene mutations of entire coding sequences in BNP occur in HCM patients without heart failure.

The aims of the present study were to investigate the gene mutations of exons 3 and 18 in MYH7, of exon 9 in TNNT2, and of all three exons (including entire coding sequences) in BNP in Chinese familial HCM patients and further to assess their effect on the clinical features of these patients.

2. Materials and methods

2.1. Clinical evaluations

Five unrelated Chinese families were studied. A clinical investigation was carried out and blood samples for DNA analysis were obtained from patients and other family members after they had given informed written consent. All subjects were evaluated based on patient and family histories, physical examinations, an electrocardiogram, and two-dimensional and Doppler echocardiography. All echocardiograms were done by the same two investigators who did not know the results of the other clinical examinations and genetic analysis. Echocardiographic evaluation was performed with an HDI 5000 Ultrasound System (USA). Measurements obtained during three consecutive cardiac cycles were averaged. The clinical diagnosis of HCM was based on: (a) ECG criteria: Q waves >0.04 second in duration and/or >1/3 of the ensuring R wave in depth and present in at least two leads, or LVH; and/or (b) echocardiographic criteria: the presence of asymmetric left ventricular hypertrophy (maximal wall thickness of septum >13 mm) in the absence of other known causes, such as hypertension and aortic stenosis [12]. Confirmation was also made in one patient by magnetic resonance imaging since echocardiography had confirmed HCM in his parents. The control group consisted of 53 healthy and age-matched volunteers who showed no abnormalities on physical and electrocardiographic examinations. The study was approved by our hospital's Institutional Review Board.

2.2. Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes using the Puregene DNA Isolation Kit from Gentra Systems (Minneapolis, MN, USA). The primers of the exons 3 and 18 in MYH7 were designed according to the reported sequences of MYH7 [13]. The primers of the exon 9 in TNNT2 and of all three exons in BNP were designed from the published sequences [14,15]. The BNP gene consists of 1992 bp and the nucleotide positions of all three exons in BNP are closed. Consequently, the entire fragment (including exon 1, exon 2, and exon 3) was amplified by the primers listed in Table 1. The primers for sequencing the BNP gene were designed as follows: exon 1 and exon 2 down: CGATGTCCAGGTGACCTTTT; exon 3 up: TCAAAGGCAGAGAGCAGGAT. Each of the exons in the genes indicated above (MYH7, TNNT2, and BNP) was amplified with the polymerase chain reaction (PCR) in a GeneAmp PCR system 9700 (Perkin-Elmer, CA) as shown in Table 1. The PCR products were visualized on 1% agarose gel by electrophoresis and purified with the PCR

Table 1
Primer sequence and PCR conditions

Gene	Position map	PCR fragment	Primers	PCR conditions
MYH7 exon				
3	5706–5950	273 bp	F 5′ACTCCAGGCACAGCCATGGG 3′ R 5′GTGGACTCTCACATCAGCCT 3′	94 °C 30″, 64 °C 30″, 72 °C 30″ (40 cycles), 72 °C 5′
18	12,401–12,874	473 bp	F 5′TCTCTCCTCTTTCCCTTCTGTCTC 3′ R 5′TGCCTTACACATCACCCTCATTAC 3′	94 °C 30″, 60 °C 30″, 72 °C 30″ (40 cycles), 72 °C 5′
TNNT2 exon				
9	13,028–13,418	390 bp	F 5′CTAGCCCACCCATCTCTCC 3′ R 5′GTCAAGGAGCATCCAGTAGG 3′	94 °C 30″, 58 °C 30″, 72 °C 1′30″ (40 cycles), 72 °C 5′
BNP[a] *exon*				
1	507–1700	1193 bp	F 5′AGGTGTCTGCAGCCAGGACT 3′	94 °C 1″, 59 °C 30″,
2	507–1700	1193 bp		
3	507–1700	1193 bp	R 5′AGCAGCAGCAGCAGCAGAAG 3′	72 °C 1′30″ (40 cycles), 72 °C 5′

[a] All three exons in the BNP gene were included in the entire PCR fragment.