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Original article

Detection of mutations in symptomatic patients with hypertrophic cardiomyopathy in Taiwan

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ABSTRACT

Background: Hypertrophic cardiomyopathy (HCM) is a common genetic cardiac disorder associated with sudden death, heart failure, and stroke. The aim of the present study was to evaluate the prevalence and types of mutations in symptomatic patients with HCM in Taiwan.

Methods: Thirty-eight HCM index patients (mean age 60 ± 16 years) underwent systematic mutation screening of eight sarcomeric genes: β-myosin heavy chain (*MYH7*), myosin-binding protein C (*MYBPC3*), troponin T (*TNNT2*), troponin I (*TNNI3*), myosin ventricular regulatory light chain 2 (*MYL2*), myosin ventricular essential light chain 1 (*MYL3*), α-tropomyosin (*TPM1*), and cardiac α-actin (*ACTC*), using direct DNA sequencing. In silico programs predicted damaging amino acids. In the positive families, genotype–phenotype correlation studies were done.

Results: Overall, 13 mutations were identified in 13 index patients (34.2%). The three most frequently mutated genes were *MYH7*, *MYBPC3*, and *TNNT2*. One patient carried double mutations. Five mutations (*MYH7* R147S; *MYBPC3* R597Q; *MYBPC3* W1007R; *TNNI3* E124Q; *MYL3* R63C) were novel; all were missense mutations. Analysis using in silico tools showed near consensus to classify these five novel mutations as pathological. Family pedigree analysis showed the presence of cosegregation in at least two affected members in each proband family, but incomplete penetrance in young family members with a positive genotype.

Conclusions: We identified 13 HCM pedigrees, including 5 carrying novel mutations and 1 with a double mutation. The three most commonly mutated genes were *MYH7*, *MYBPC3*, and *TNNT2*. These results, together with genetic counseling, could lead to earlier diagnosis and better management of family members at risk of HCM.

Introduction

Hypertrophic cardiomyopathy (HCM), clinically defined as thickening of the myocardial wall in the absence of any other cause of left ventricular (LV) hypertrophy, is often inherited genetically, and affects 1:500 individuals [1,2]. The clinical and pathological manifestations are diverse and they range from asymptomatic clinical courses to severe heart failure and sudden cardiac death (SCD). It is caused by 11 or more genes encoding proteins of the cardiac sarcomere [3]. From patients who have been genotyped successfully, around 70% have been found to have mutations in the gene encoding β-myosin heavy chain (*MYH7*) or myosin-binding protein C (*MYBPC3*). Troponin T (*TNNT2*) and several other genes account for 5% or less of cases. Existing data have been largely obtained for Caucasian samples. However, no data have been derived from a systematic screening of HCM from the Taiwanese, who comprise the major population group in Taiwan and are the descendants of early settlers from the southeast coast of China during the past 400 years or more [4,5]. Although there have been a few systematic surveys of gene-proven HCM from Chinese [6,7], the relationships need to be further investigated. Therefore, we investigated the prevalence and type of mutations among unrelated Taiwanese with symptomatic HCM. In all patients, a systematic screening for mutations was performed in eight genes that code for the components of the

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sarcomere: *MYH7*, *MYBPC3*, *TNNT2*, troponin I (*TNNI3*), myosin ventricular regulatory light chain 2 (*MYL2*), myosin ventricular essential light chain 1 (*MYL3*), α-tropomyosin (*TPM1*), and cardiac α actin (*ACTC*).

Materials and methods

Subjects

Unrelated symptomatic adult patients were recruited from two tertiary referral centers (Taipei and Kaohsiung Veterans General Hospitals, Taiwan). Informed consent was obtained from all the patients according to institutional guidelines. The condition was diagnosed following the American College of Cardiology/European Society of Cardiology (ACC/ESC) criteria, using inclusion criteria of a left ventricular wall thickness ≥13 mm on echocardiography when no other cause explained the hypertrophy [8]. The 12-lead electrocardiograms obtained at or near the time of initial HCM diagnosis were assessed. LV hypertrophy by electrocardiographic criteria was adopted by the Sokolow–Lyon criteria: the sum of S1 wave in V1 and R wave in V5 (or V6) >38 mm [9]. Strain pattern was characterized by tall lateral precordial voltages in association with ST–T abnormalities in leads V5 and V6. Prognosis in families was assessed at the time of genotyping and was based on family history. A major cardiac event was defined as sudden death, heart failure death, stroke death, or resuscitated death related to HCM, each occurring before 60 years of age. Probands with any relative diagnosed with HCM were considered familial cases, and patients with proven HCM but without familial history or affected relatives were considered sporadic cases.

Genetic study

Genomic DNA was isolated from the leukocytes of the peripheral blood of the patients. Polymerase chain reaction was used to amplify the exons and flanking intronic bases of the eight genes, including *MYH7* (40 exons), *MYBPC3* (35 exons), *TNNT2* (17 exons), *TNNI3* (8 exons), *MYL2* (7 exons), and *MYL3* (6 exons). When no mutation was found, analysis of the *TPM1* (nine exons) and *ACTC* (six exons) genes was performed. The primers used for the polymerase chain reaction were designed using reference sequences deposited in the GenBank database. Information on the primers and amplification conditions can be obtained from the authors at the correspondence address. Standard DNA sequencing reactions were performed using the fluorescence-labeled dideoxy chain termination method with the Big Dye Terminator ABI Prism Kit and the ABI PRISM™ 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

Confirmation of mutation and family genotyping

A variant was considered a mutation in accordance with the following criteria: (1) presence in tested affected members of the family of a proband; (2) absence from 200 unrelated chromosomes of the control subjects; (3) absence from a public database of polymorphisms, the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/); (4) conservation of the mutated residue among species and isoforms; and/or (5) the gene has been reported as an HCM-causing mutation in the literature. Moreover, the variants were revised to assess their pathogenicity using in silico tools. The topological placement of the mutations was localized using the SwissProt database (http://ca.expasy.org/uniprot/) and the bibliography previously described [10]. The UniProt database provides generally accepted residue ranges corresponding with each domain region and specialized subregion. To predict the pathogenicity of the damaging amino acid substitution, four online tools, SIFT (http://sift.jcvi.org/www/SIFT_seq_submit2.html), Pmut (http://mmb.pcb.ub.es/PMut/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and SNAP (http://www.rostlab.org/services/snap/), were used. A missense mutation was assumed to be possibly disease-causing if at least two independent programs indicated a damaging effect. To predict the altered reading frame in the nonsense mutation, the online software Open Reading Frame (ORF) Finder from the NCBI (http://www.ncbi.nlm.nih.gov/projects/gorf/) was used. Family members of the probands with the identified mutations were invited to participate in this investigation, regardless of whether they had symptoms of the disease.

Statistical analysis

The data for continuous variables have been expressed as mean value with ranges and compared using the non-parametric Kruskal–Wallis *H* test. The data for categorical variables have been expressed as numbers or percentages and compared using Fisher's exact test. Data were collected and analyzed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). A *p*-value of less than 0.05 was considered statistically significant.

Results

Clinical characteristics and genetic results

The clinical characteristics of all patients are summarized in Table 1. There were no significant differences in sex, symptom severity, LV wall thickness, and presence of LV outflow tract (LVOT) obstruction for the genotype-positive patients and those without mutations, but genotype-positive patients had an earlier age of onset at diagnosis and higher incidence of family history of SCD. The enrolled patients with LVOT >50 mmHg and refractory medications (44.7%) underwent alcohol septal ablation later. Overall, 13 mutations in the selected genes were identified in 13 patients, leading to a genetic diagnosis in 34.2% of the index patients (Table 2). Five mutations were novel.

The genes most frequently involved in the genotype-positive patients were *MYH7* and *MYBPC3*, which were mutated in 46.2% and 30.8% patients, respectively. The other mutated genes (*TNNT2*, *TNNI3*, and *MYL3*) were involved in 30.8% of cases. The distribution of the different mutation types was 92.3% missense ($n = 12$), and 7.7% deletions ($n = 1$). One patient carried double mutations, with a mutation in *MYH7* R858C and another in *TNNT2* R286H. Table 3 shows the echocardiographic and electrocardiographic findings at age of diagnosis in index patients carrying mutations.

To determine the pathogenicity of these mutations, we performed an in silico study and surveyed the family pedigree. Table 4 shows a suite of different tools. The PolyPhen structure-based method predicted that all changes were damaging. In contrast, *MYH7* R663H, E1902Q were identified as a neutral variant according to SNAP; *MYH7* E1902Q, *MYBPC3* Q998E and *TNNI3* E124Q were identified as a neutral variant according to Pmut. Five mutations were novel: one mutation in *MYH7*, two in *MYBPC3*, one in *TNNI3*, and one in *MYL3*. All are missense mutations. Analysis using SIFT, PolyPhen, SNAP, and Pmut showed near consensus for classifying the five novel mutations as pathological.

Pedigree and mutations analysis of these five novel and the double families

Fig. 1 shows the pedigrees of all the families with novel mutations and the double mutation. A person with Proband *MYH7* R147S experienced chest pain at the age of 48 years. Echocardiography showed asymmetric septal hypertrophy (ASH) with mid LV