

## Hypertrophic Cardiomyopathy: Infrequent Mutation of the Cardiac Beta-Myosin Heavy-Chain Gene

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The aim of this study was to identify mutations in the cardiac heavy-chain beta-myosin gene (*MYH7b*) in a group of Spanish patients with hypertrophic cardiomyopathy. The study included 36 families with at least one member who had hypertrophic cardiomyopathy. DNA from exons 3 to 24 of the *MYH7b* gene was sequenced. Two mutations were identified: Arg858Cys and Met515Val. They occurred in 2 families, one of which was of Moroccan origin. This corresponds to a *MYH7b* gene mutation frequency of less than 5%. In contrast to findings in other Caucasian populations, *MYH7b* gene mutation occurred infrequently in this group of Spanish families with hypertrophic cardiomyopathy.

**Key words:** Hypertrophy cardiomyopathy. *MYH7b* gene mutation.

### Miocardiopatía hipertrófica: baja frecuencia de mutaciones en el gen de la cadena pesada de la betamiosina cardíaca

El objetivo del presente estudio es la búsqueda de mutaciones en el gen de la cadena pesada de la betamiosina cardíaca (*MYH7b*) en pacientes españoles con miocardiopatía hipertrófica. Se incluyó en el estudio a 36 familias con al menos un individuo enfermo de miocardiopatía hipertrófica. Se secuenció el ADN de los exones 3 al 24 del gen de la cadena pesada de la betamiosina cardíaca (gen *MYH7b*). Se ha identificado 2 mutaciones (Arg858Cys y Met515Val) en 2 familias distintas, una de ellas de origen marroquí, lo que representa una frecuencia de mutaciones en el gen *MYH7b* inferior al 5%. Al contrario de lo que ocurre en otras poblaciones caucásicas, las mutaciones en el gen *MYH7b* en este grupo de familias españolas con miocardiopatía hipertrófica son poco frecuentes.

**Palabras clave:** Miocardiopatía hipertrófica. Mutaciones en *MYH7b*.

## INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a heart disease characterized by ventricular hypertrophy, generally of the left ventricle. Usually, the interventricular septum is strongly affected. No cause

of such hypertrophy is immediately apparent. The prevalence of this disease is 1:500-600,<sup>1,2</sup> depending on the population. Inherited in the Mendelian fashion (autosomic dominant), the severity of the problem depends upon the mutation carried.

Twelve genes associated with this disease have been identified, of which nine code for proteins of the cardiac sarcomere.<sup>3-10</sup> In total, more than 150 different mutations are thought to lie at the root of the problem. Studies of large numbers of patients of European and North American origin show mutations of the cardiac beta-myosin heavy-chain gene (*MYH7b*) to be that most commonly associated with HCM; some 20%-30% of all patients with this disease have a mutation of this gene.

The aim of the present study was to analyze the sequence of *MYH7b* in a number of Spanish patients

This work was partially funded by a *Realización de Proyectos Coordinados de Investigación en Ciencias de la Salud de la Comunidad de Madrid* grant (08.4/0035/98), and by a grant from the *Fundación MAPFRE Medicina 2005* (cardiovascular research).

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Received July 20, 2005.

Accepted for publication January 26, 2006.

with HCM to establish the frequency and the types of mutation shown by this gene in Spain.

## METHODS

### Patients

The study subjects were 144 patients belonging to 36 families, each with at least one member who suffered HCM. The diagnostic criteria for inclusion in the study were: a left ventricular wall or interventricular septum of thickness  $\geq 13$  mm (in adults) with no immediately apparent cause of such hypertrophy<sup>11</sup>; in the direct family members of affected patients, mild ventricular hypertrophy (thickness  $\leq 13$  mm) associated with an abnormal electrocardiogram (ECG; abnormal Q wave or marked inversion of the T wave) was deemed sufficient for a positive diagnosis to be made. The patients analyzed (24 males and 12 females aged 3-79 years) were recruited at the cardiology units of hospitals in Madrid. Thirty three of the corresponding families were Spanish, 3 were Moroccan of Arabic origin. All patients had undergone at least one ECG at some time. All patients gave their informed consent to be included, in keeping with the demands of the ethics committee of the Hospital Universitario La Paz.

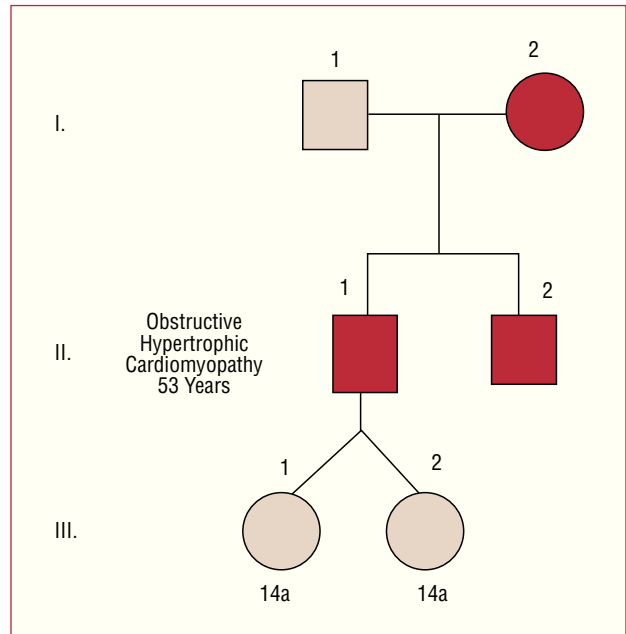
### Genetic Analyses

The *MYH7b* gene was sequenced in one member of each family. Exons 3-24 (both inclusive) and the adjacent intronic regions were amplified separately by the polymerase chain reaction (PCR). Both DNA strands were sequenced following the Sanger method. The resulting sequences were compared with those for *MYH7b* stored in NCBI database (<http://www.ncbi.nlm.nih.gov/entrez>) (ref. NT\_026437).

In those families in which more than 1 member could be studied, 2 *MYH7b* intragene microsatellite markers, MYO I and MYO II,<sup>12</sup> were analyzed to determine whether a mutation of this gene was the cause of disease in each patient.

## RESULTS

Eighteen of the patients had a family history of HCM and 18 showed sporadic presentation. Analysis of the polymorphic markers MYO I and MYO II showed that the mutation of *MYH7b* was not the cause of the disease in 6 families. In the remaining 30 patients the disease either appeared sporadically or the number of individuals in each family was too small to allow the segregation of alleles to be analyzed and correlated to clinical manifestations. In these 30 patients, the regions of the gene where the mutations that most commonly cause HCM were analyzed.<sup>13</sup>



**Figure 1.** Family 3 of patient AF, a carrier of the R858C (Arg858Cys) mutation in gene *MYH7b*.

Sequencing from exon 3 to exon 24 in these subjects allowed 2 mutations to be identified in 2 different families. One of the patients (AF) was a heterozygous carrier of the Arg858Cys mutation which causes a change in the net charge of the coded protein from +1 to 0 (it was not seen in 100 corresponding chromosomes from healthy individuals). This patient, who was 53 years old, suffered familial, obstructive HCM (Figure 1; subject II.1). His mother and brother were also afflicted. The other mutation found was Met515Val. This exact mutation has not been previously described, although very similar mutations have been reported,<sup>14</sup> even affecting the same aminoacid.<sup>15</sup> This patient, a 27 year-old man of Moroccan origin, suffered non-obstructive asymmetric septal hypertrophy (septum thickness 27 mm) and repeated syncope.

## DISCUSSION

Hypertrophic cardiomyopathy is frequently associated with mutations of *MYH7b*, a gene that codes for the heavy chain of cardiac beta-myosin. Sequencing of more than 80% of this gene, including the regions where mutations most commonly occur, identified 2 mutations in 36 families (5.5%). One of these mutations, Arg858Cys, has been described by van Driest et al,<sup>15</sup> although these authors provide no clinical data for the patient involved. Aminoacid Arg858 has been conserved throughout evolution, reflecting its functional importance (Figure 2B). The



**Figure 2.** Aminoacid sequence of the heavy chain of human cardiac beta-myosin, showing the conservation of methionine 515 (A) and arginine 858 (B).

other mutation detected was Met515Val. The strict conservation of methionine 515 (Figure 2A) indicates that this mutation may affect the function of the protein.

The present results show that mutations of *MYH7b* in the Spanish population of patients with HCM is uncommon compared to that seen in other Caucasian groups. As in other studies of patients with HCM, the present population was recruited at hospital clinics, therefore a selection bias with respect to the general population of patients with HCM (many of whom are undiagnosed) cannot be ruled out. The results of this study are of importance with respect to diagnostic strategies and in the genetic counseling of families affected by this disease.

## ACKNOWLEDGEMENTS

We thank the families who took part in this work, B. Ejarque for help with DNA purification, D. Arjona and C. Amiñoso for their help with DNA sequencing, and M.J. Mazón for critical reading of the manuscript. We also thank the many medical professionals involved in the clinical study of the patient population.

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