Genetic variations of $\beta$-MYH7 in Venezuelan patients with hypertrophic cardiomyopathy.

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Keywords: MYH7, sudden death, hypertrophy, cardiomyopathy, mutation.

Abstract. Hypertrophic cardiomyopathy (HCM) is a cardiac disease, characterized by marked hypertrophy and genetic variability. HCM has been associated with sarcomere protein mutations, being cardiac $\beta$-myosin (coded by the MYH7 gene) and myosin binding protein C (coded by the MYBPC3 gene) the most frequently affected proteins. As in Venezuela only the clinical analysis are performed in HCM patients, we decided to search for genetic variations in the MYH7 gene. Coding regions, including the junction exon-intron of the MYH7 gene, were studied in 58 HCM patients, whose samples were collected at the ASCARDIO Hospital (Barquisimeto, Lara state, Venezuela) and 106 control subjects from the ASCARDIO Hospital and the IVIC (Barquisimeto Lara state and Miranda, Venezuela, respectively). The blood samples were analyzed by genomic DNA isolation, followed by polymerase chain reaction and sequence analysis. The screening of the MYH7 gene revealed eight already reported polymorphic variants, as well as two intronic variations in these HCM patients. Neither any missense mutations nor other pathological mutations in the MYH7 gene were found in the HCM patients. 
Variaciones genéticas en el gen MYH7 en pacientes venezolanos con miocardiopatía hipertrófica.


Palabras clave: MYH7, muerte súbita, hipertrofia, miocardiopatía, mutación.

Resumen. La miocardiopatía hipertrófica (MNH) es una enfermedad cardíaca primaria, caracterizada por una marcada hipertrofia y variabilidad genética. MH ha sido asociada con mutaciones en las proteínas del sarcómero, siendo la beta miosina cardíaca, codificada por el gen MYH7 y la proteína de unión a miosina C, codificada por el gen MYBPC3, las principalmente afectadas. En Venezuela únicamente se realiza el diagnóstico clínico de MH, por lo cual el objetivo principal de este trabajo fue realizar el análisis genético en los pacientes, iniciando con el gen MYH7. Para ello, se estudió la región codificante, incluyendo la región de unión exón-intron del gen MYH7 en 58 pacientes provenientes de ASCARDIO (Barquisimeto, estado Lara) y 106 controles provenientes de ASCARDIO e IVIC (estados Lara y Miranda, Venezuela). Se colectaron las muestras de sangre para el aislamiento del ADN genómico, se realizó la técnica de PCR, seguido del análisis de secuencias para la detección de mutaciones en pacientes y controles. Se encontraron 8 polimorfismos previamente reportados, y 2 variaciones intrónicas. No se encontraron mutaciones que involucaran un cambio de aminoácido en ninguno de los exones del gen MYH7 de la beta miosina cardíaca.


INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is defined as a primary cardiac disease (1, 2) characterized by left ventricular hypertrophy in the absence of any other disease (they can be cardiac or systemic) that can lead to a secondary hypertrophy. Histologically, there are structural hypertrophy and myocyte disarray with interstitial fibrosis (3-5). HCM is the leading cause of sudden death in young people and athletes (6). HCM affects 1 in 500 people worldwide (3-5). It is mainly caused by mutations in genes encoding sarcomere proteins (7-10). Presently, there are approximately 1400 mutations reported in 23 HCM related genes (11).

Mutations in two genes-β-myosin heavy chain (MYH7) and myosin binding protein C (MYBPC3, cardiac isoform) are responsible for 50 to 70% of genetic cases of HCM (3, 12, 13). β-myosin, a large 1935 amino acid protein located on the long arm of human chromosome 14, specifically 14q11. 2-q13, interacts with the thin filament during muscle contraction. The gene consists of 40 exons which produce a transcript of 6,027 bp (5, 14). Myosin is a hexameric protein that consists of two myosin heavy chains and two pairs of non-identical light chains, the regulatory (RLC) and essential (ELC) light chains (15, 7). The β-myosin heavy chain is divided into three regions: the subfragment 1 (S1), subfragment 2 (S2) and light meromyosin (LMM) (Fig. 1) (15, 5). More than 200 different mutations
have been identified in HCM with respect to the MYH7 gene (16) and most of them are found in the S1 and S2 regions. The mutations have been associated with marked hypertrophy and severe clinical phenotype (4, 17), although the frequency of mutations found in the MYH7 gene is variable and depends on the type of study (18).

There are no published studies regarding mutations on clinical cases of hypertrophic cardiomyopathy in Venezuela. In this study, we analyzed the coding region including the intron-exon boundaries of the MYH7 gene corresponding to patients diagnosed with HCM to establish the frequency and possible types of mutations shown by this gene in the Venezuelan population.

**PATIENTS AND METHODS**

**Patients**

The study was performed between 2010-2012 on 58 individuals genetically independent, between 13 and 70 years of age, from Lara state, Venezuela and diagnosed at ASCARDIO Hospital with HCM by physical examination, electrocardiogram, echocardiogram, and holter (Table I). The control samples were collected from 106 healthy blood donors from ASCARDIO Hospital and IVIC, with no history of heart disorders. The diagnostic criteria for inclusion in the study were: a left ventricular wall thickness or interventricular septum = 13 mm, with no cause of such hypertrophy, as well as the characteristic symptoms corresponding to HCM pathology as revealed by the echocardiograms. All subjects gave their informed consent to be included in this study, which was approved by the ASCARDIO and IVIC ethics committee.

**TABLE I**

<table>
<thead>
<tr>
<th>Description</th>
<th>Patients (n=58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of diagnosis (years)</td>
<td></td>
</tr>
<tr>
<td>13-22</td>
<td>12</td>
</tr>
<tr>
<td>26-49</td>
<td>28</td>
</tr>
<tr>
<td>51-70</td>
<td>18</td>
</tr>
<tr>
<td>Male sex</td>
<td>30</td>
</tr>
<tr>
<td>Female sex</td>
<td>28</td>
</tr>
<tr>
<td>Arterial Hypertension</td>
<td>11</td>
</tr>
<tr>
<td>NYA I</td>
<td>3</td>
</tr>
<tr>
<td>NYA II</td>
<td>19</td>
</tr>
<tr>
<td>NYA III</td>
<td>8</td>
</tr>
<tr>
<td>Chest Pain</td>
<td>16</td>
</tr>
<tr>
<td>Syncope</td>
<td>7</td>
</tr>
<tr>
<td>Shortness of breath (dyspnea)</td>
<td>30</td>
</tr>
<tr>
<td>Altered state of consciousness</td>
<td>10</td>
</tr>
</tbody>
</table>
Methods

Genetic analyses: DNA was isolated from samples of peripheral blood using the protocol described by Lahiri and Nurnberger (19). The coding sequences of the MYH7 gene were amplified with PCR using genomic DNA, with primers reported in: http://www.cardiogenomics.com/. Polymerase chain reaction was carried out in 0.5 mL tubes. Each tube contained 10-20 ng of genomic DNA, 0.2 mM each of forward and reverse primer, 0.5-1 U of taq DNA polymerase enzyme, 0.2 mM of dNTP, 5 µL PCR buffer and water to make up the final volume to 50 µL.

Amplification was carried out with the annealing temperature varying from 58°C to 62°C (based on the exon). Amplified fragments were purified with the AxyPrep™ Blood Genomic DNA Miniprep Kit and sent to Macrogen (Korea) for direct sequencing. The sequences obtained were analyzed using the software McVector (Version 11.1.2) and compared with the sequences stored in the NCBI database (http://www.ncbi.nlm.nih.gov/entrez) of the MYH7 gene (NP_000248.2).

The changes observed in the sequences were confirmed by four additional sequence analyses from independent PCR reactions. All the possible mutations were validated by bi-directional DNA sequencing. Each of the possible mutations in this study were checked if present in the NCBI database on line or published. Additionally, we defined pathogenic mutations as those that were reported to be present in HCM patients, and not in healthy relatives. We also considered a mutation as pathogenic when the affected amino acid was in regions conserved among species. A polymorphism was defined as a change in nucleotide sequence present in control individuals or sequences previously reported in the database on line. (http://www.ncbi.nlm.nih.gov/SNP/; http://www.cardiogenomics.org, http://www.hgmd.org, and http://swissvar.expasy.org/).

RESULTS

In this study we report the screening of possible mutations of the MYH7 gene coding regions including the intron-exon boundaries, and not only exons coding for the head motor domain of the protein. We found 8 variants, reported as polymorphisms (Table II), and 2 intronic variations (Table III). We did not identify any missense mutations in the MYH7 gene in the HCM patients.

The analysis of exon 16 revealed the following changes: 4 patients and 5 controls exhibited the allele A for the polymorphism Glu535Glu (20), while only 1 control individual exhibited the C allele for the position 585. Three patients had the allele C for the Asn589Asn polymorphism.

In exon 12, we detected only one patient with the allele G for the Lys 365 Lys polymorphism (20-22) in homozygosis, and 7 patients plus 18 controls heterozygotes. Additionally, two patients and one control with the allele C for the Asp 376 Asp polymorphism (20) in homozygosis, and 19 controls plus 9 patients heterozygotes for the same polymorphism in exon 12.

The most common genetic variations were found in exon 23. In this study all the controls evaluated showed the allele T, in its heterozygous form, for the polymorphisms Ala 917 Ala and Leu 943 Leu, while only 29 out of 58 HCM patients showed the same changes.

In exon 3, 81 controls and 22 patients had the allele T for the Thr 63 Thr polymorphism (20-22), only four patients were homozygous, while the rest of individuals were heterozygous. On this study, only 1 patient carried 6 polymorphic variants, excluding the change Ile 585 Ile (Table II).
The sequencing results additionally revealed two genetic variants, located in two intronic regions: a transition C > T in intron 15 found in only 3 controls; a substitutions in intron 19 (-17 A>G) which was found in 55 controls plus 20 patients (Table III). All individuals were heterozygotes. Therefore, only polymorphisms in the MYH7 gene were found. We did not find any missense mutations or nonsense mutations. This could be probably due to their presence in other genes that might be responsible for the clinical symptoms observed, as all patients showed left ventricular hypertrophy consistent with the clinical features of HCM.

TABLE II
**MYH7 GENE POLYMORPHISM FOUND IN VENEZUELAN HCM PATIENTS**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Gen position</th>
<th>Protein position</th>
<th>Control/Patients N=106/58</th>
<th>Allelic frequency (control)</th>
<th>Allelic frequency (Patients)</th>
<th>SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3</td>
<td>g.5909 T&gt;C</td>
<td>Thr 63 Thr</td>
<td>81/22</td>
<td>T=0.382</td>
<td>C=0.618</td>
<td>rs2069540</td>
</tr>
<tr>
<td>E12</td>
<td>g.9633 G&gt;A</td>
<td>Lys 365 Lys</td>
<td>18/8</td>
<td>G=0.084</td>
<td>A=0.915</td>
<td>rs735711</td>
</tr>
<tr>
<td>E12</td>
<td>g.9666 C&gt;T</td>
<td>Asp 376 Asp</td>
<td>20/11</td>
<td>C=0.099</td>
<td>T=0.900</td>
<td>rs2231126</td>
</tr>
<tr>
<td>E12</td>
<td>g.11573A&gt;G</td>
<td>Glu 535 Glu</td>
<td>5/4</td>
<td>A=0.023</td>
<td>G=0.976</td>
<td>rs2069543</td>
</tr>
<tr>
<td>E16</td>
<td>g.11723 C&gt;A</td>
<td>Ile 585 Ile</td>
<td>1/0</td>
<td>C=0.004</td>
<td>A=0.995</td>
<td>rs201860580</td>
</tr>
<tr>
<td>E16</td>
<td>g.11735 C&gt;T</td>
<td>Asn 589 Asn</td>
<td>0/3</td>
<td>C=0</td>
<td>T=1</td>
<td>rs3729816</td>
</tr>
<tr>
<td>E16</td>
<td>g.15354 T&gt;C</td>
<td>Ala 917 Ala</td>
<td>106/29</td>
<td>T=0.5</td>
<td>C=0.5</td>
<td>rs1041957</td>
</tr>
<tr>
<td>E23</td>
<td>g.15430 T&gt;C</td>
<td>Leu 943 Leu</td>
<td>106/29</td>
<td>T=0.5</td>
<td>C=0.5</td>
<td>rs2856898</td>
</tr>
</tbody>
</table>

SNP: Single nucleotide polymorphism.

TABLE III
**MYH7 GENE INTRONIC VARIATIONS FOUND IN VENEZUELAN HCM PATIENTS**

<table>
<thead>
<tr>
<th>Position in the gene</th>
<th>Intron</th>
<th>Change in the nucleotide sequence</th>
<th>Control/Patients N=106/58</th>
<th>Allelic Frequency (Controls)</th>
<th>Allelic Frequency (Patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>g.12721</td>
<td>IVS15-256</td>
<td>C&gt;T</td>
<td>3/0</td>
<td>C=0.014</td>
<td>C=0</td>
</tr>
<tr>
<td>g.14788</td>
<td>IVS19-17</td>
<td>A&gt;G</td>
<td>55/20</td>
<td>A=0.259</td>
<td>A=0.172</td>
</tr>
</tbody>
</table>

IVS: Intervening sequence N= Number of Control and Patients.
DISCUSSION

This study is the first report on the mutation frequency of the MYH7 gene in a population of patients clinically diagnosed with HCM in Venezuela. The MYH7 gene was the first gene associated with HCM in humans (23), and it has been reported that MYH7, together with MYBPC3—the gene encoding cardiac isoform of myosin-binding protein C—, are responsible for about 70% of genotyped HCM cases (24). In Venezuela, HCM patients are diagnosed exclusively by clinical evaluation because genetic trials are not available. It is therefore important to examine the genetic background in those individuals already diagnosed with HCM. The present study constitutes an important starting point which should lead to a better understanding of the genetic basis of HCM in Venezuela, and also contribute to the development of valuable diagnostic tools for identifying individuals of the patients family who are at risk for HCM. Examining all of the genes in which disease associated mutations have been described would entail considerably more resources and technology than the ones currently available.

The mutations that have been described in the MYH7 gene are predominantly missense mutations, located principally in the globular myosin head (25-27). There are also mutations, described in the rod region of the gene (5), that have been associated largely with dilated cardiomyopathy (5).

Here we report eight polymorphisms in exons and two genetic variations in intronic regions. In our studied population sample, we did not find any missense mutations in the MYH7 gene. The absence of missense mutations on these clinically diagnosed HCM patients, has several explanations. First, the population studied consisted of only clinically diagnosed patients not previ-
The clinical heterogeneity is demonstrated by instances where two individuals from the same family share the same mutation but exhibit different symptomatology; or even cases where one individual remains asymptomatic while others with the same mutation develop clinical symptoms early in life, including cardiac failure, or severe arrhythmia (31).

According to Golbus et al. (33) the MYH7 gene compared to MYBPC3 and TNN, the gene that codes for Titin, has fewer protein-altering variation (PAV), such as missense or nonsense mutation polymorphisms, insertion/deletions in the coding regions and splice site altering variants. Therefore, it is not strange the absence of mutations found in the small group of patients studied in this research. In conclusion, the cardiac β-myosin heavy chain gene is not the predominant gene for hypertrophic cardiomyopathy in Venezuelan patients.

ACKNOWLEDGMENTS

We thank Dr. Raúl Padrón, for his comments on this work, Marinela Falcone MD, for the sending of blood samples, Dr. Gustavo Márquez, and Lic. Daniel Romero for their help with the manuscript.

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