Somatic MYH7, MYBPC3, TPM1, TNNT2 and TNNI3 Mutations in Sporadic Hypertrophic Cardiomyopathy

Lucía Núñez, PhD; Juan Ramón Gimeno-Blanes, MD, PhD; María Isabel Rodríguez-García, BSc, PhD; Lorenzo Monserrat, MD, PhD; Esther Zorio, MD; Caroline Coats, BSc; Christopher G. McGregor, MD; Juan Pedro Hernandez del Rincón, MD, PhD; Alfonso Castro-Beiras, MD, PhD; Manuel Hermida-Prieto, BSc, PhD

Background: Hypertrophic cardiomyopathy (HCM) is a clinically heterogeneous genetic heart disease characterized by left ventricular hypertrophy in the absence of another disease that could explain the wall thickening. Elucidation of the genetic basis of HCM lead to the identification of several genes encoding sarcomeric proteins, such as MYH7, MYBPC3, TPM1, TNNT2, and TNNI3. Sarcomeric genes are mutated in approximately 40% of HCM patients and a possible explanation for the incomplete yield of mutation-positive HCM may be somatic mutations.

Methods and Results: We studied 104 unrelated patients with non-familial HCM. Patients underwent clinical evaluation and mutation screening of 5 genes implicated in HCM (MYH7, MYBPC3, TPM1, TNNT2, and TNNI3) in genomic DNA isolated from resected cardiac tissue; 41 of 104 were found to carry a mutation, but as several patients carried the same mutations, the total amount of different mutations was 37; 20 of these mutations have been previously described, and pathogenicity has been assessed. To determine the effect of the 17 new mutations an in silico assay was performed and it predicted that 4 variants were damaging mutations. All identified variants were also seen in the DNA isolated from the corresponding blood, which demonstrated the absence of somatic mutations.

Conclusions: Somatic mutations in MYH7, MYBPC3, TPM1, TNNT2, and TNNI3 do not represent an important etiologic pathway in HCM. (Circ J 2013; 77: 2358–2365)

Key Words: Hypertrophic cardiomyopathy; MYBPC3; MYH7; Somatic mutations

Hypertrophic cardiomyopathy (HCM) is a heterogeneous genetic disease characterized by the development of left ventricular hypertrophy in the absence of abnormal loading conditions that could explain that it. It has a frequency of 0.2% in the adult population and is a major cause of sudden cardiac death (SCD) in young people (<35 years old). HCM is regarded as a disease entity caused by autosomal dominant mutations in genes encoding protein components of the sarcomere and its constituent myofilament elements. The current weight of evidence supports the view that the vast majority of genes and mutations responsible for clinically diagnosed HCM encode proteins within and associated with the sarcomere, responsible for generating the molecular force of myocyte contraction, accounting in a large measure for those patients described in the voluminous amount of HCM literature published over 50 years (>1,400 mutations identified). However, genetic testing for these genes led to identification of disease-causing mutations in 30–62% of unrelated HCM patients across the various cohorts tested around the world. That is, if all sarcomeric genes were screened, on the basis of published data, approximately 40% of patients would have a genotype that was negative for sarcomeric mutation. These patients could have a mutation in a non-sarcomeric protein; in fact, mutations in GLA, GAA, LAMP2, and PRKAG2 have been described, but these mutations associated to HCM are rare and in most cases are associated with other syndromes. Another possible explanation for the incomplete yield of mutation-positive HCM may be somatic mosaicism. Somatic mosaicism refers to the condition in which a muta-
somatic mutations in HCM arise after fertilization such that only a subset of cells or tissues harbors the defect. Aside from occupying a major role in the pathogenesis of many cancers, somatic mosaicism has been shown to underlie some cases of certain genetic disorders. Moreover, this genetic mechanism has been raised theoretically as relevant for several diseases in the heart as congenital heart defects and atrial fibrillation.

Taking these data together, we hypothesized that the HCM patients who are genotype-negative for sarcomeric genes also have a genetic basis because of somatic mutations. We focus on screening mutations in MYH7, MYBPC3, TNNI3, TNNT2 and TPM1, because the majority of HCM is caused by mutations in genes encoding components of the cardiac sarcomere, in genomic DNA isolated from cardiac tissue of HCM patients and its correlation with the variants in genomic DNA isolated from peripheral blood leukocytes. Moreover, the study would determine the frequency and type of mutations in the genes studied in the HCM cohort.

Methods

Patients

We included in the study 104 unrelated index cases from 9 different institutions (Complejo Hospitalario Universitario A Coruna, Spain; Hospital Virgen de la Arrixaca, Murcia, Spain; Hospital Reina Sofia, Murcia, Spain; Hospital La Fe, Valencia, Spain; Hospital General, Valencia, Spain; Hospital Puerta de Hierro, Madrid, Spain; Instituto de Medicina Legal, Valencia, Spain; Instituto de Medicina Legal, Murcia, Spain; and Heart Hospital, London, UK), all diagnosed as HCM according to the clinical and/or histological criteria of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases.

We excluded patients with a definitive diagnosis of HCM in a first-degree relative.

We obtained cardiac tissue specimens from all the patients, who had undergone endomyocardial biopsy, septal myectomy, or heart transplantation between 2009 and 2011 at the hospitals included in the study (Table 1). Moreover, all the patients provided blood samples for DNA analysis confirmation. The study was approved by the “Comité ético de investigación de Galicia” and conformed to the ethical guidelines of the 2008 Declaration of Helsinki. Informed consent was given for obtaining the samples and for the genetic screening test.

Genetic Study

Genomic DNA was isolated from cardiac tissue specimens using Illustra™ Nucleon Genomic DNA Extraction kit HT (GE Healthcare). The mutation analysis was carried out by polymerase chain reaction (PCR) followed by direct sequencing. The primers were designed using Primer Express software and the reference sequence from GenBank-GRCh37.p9 as follows: [MYH7: NC_000014.8 (23880947..23905496); MYBPC3:

<table>
<thead>
<tr>
<th>Table 1. Clinical Characteristics of Patients Included in the Study and Source of the Tissue Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>--------------------</td>
</tr>
<tr>
<td>104</td>
</tr>
</tbody>
</table>

LVOT, left ventricular outflow tract; LVH, left ventricular hypertrophy; NYHA, New York Heart Association.

Table 2. Mutations Found in MYBPC3 Gene

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Genomic position</th>
<th>cDNA</th>
<th>Exon/intron</th>
<th>Protein location</th>
<th>Patients*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A216T</td>
<td>g3921G&gt;A</td>
<td>c646G&gt;A</td>
<td>E5</td>
<td>C1</td>
<td>1</td>
</tr>
<tr>
<td>V219L</td>
<td>g5162G&gt;C</td>
<td>c655G&gt;C</td>
<td>E6</td>
<td>C1</td>
<td>2</td>
</tr>
<tr>
<td>E258K</td>
<td>g5279G&gt;A</td>
<td>c772G&gt;A</td>
<td>E6</td>
<td>Linker C1-C2</td>
<td>1</td>
</tr>
<tr>
<td>G278E</td>
<td>g6034G&gt;A</td>
<td>c833G&gt;A</td>
<td>E8</td>
<td>Linker C1-C2</td>
<td>1</td>
</tr>
<tr>
<td>IVS7+2T&gt;C*</td>
<td>g5848T&gt;C</td>
<td>c821+2T&gt;C</td>
<td>I7</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>IVS11+8C&gt;T*</td>
<td>g7084C&gt;T</td>
<td>c926+8C&gt;T</td>
<td>I11</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>IVS14−13G&gt;A</td>
<td>g10545G&gt;A</td>
<td>c1227−13G&gt;A</td>
<td>I14</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>R495W</td>
<td>g10084C&gt;T</td>
<td>c1483C&gt;T</td>
<td>E17</td>
<td>C3</td>
<td>1</td>
</tr>
<tr>
<td>R502W</td>
<td>g11005G&gt;C</td>
<td>c1504C&gt;T</td>
<td>E17</td>
<td>C3</td>
<td>1</td>
</tr>
<tr>
<td>IVS17+4A&gt;T</td>
<td>g11129A&gt;T</td>
<td>c1624+4A&gt;T</td>
<td>I17</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>IVS17+5G&gt;T*</td>
<td>g11139G&gt;T</td>
<td>c1624+5G&gt;T</td>
<td>I17</td>
<td>–</td>
<td>1</td>
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<tr>
<td>W711X*</td>
<td>g14364G&gt;A</td>
<td>c2133G&gt;A</td>
<td>E22</td>
<td>C5</td>
<td>1</td>
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<tr>
<td>IVS22−1G&gt;A*</td>
<td>g15023G&gt;A</td>
<td>c2149−1G&gt;A</td>
<td>I22</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>A833T</td>
<td>g16207G&gt;A</td>
<td>c2497G&gt;A</td>
<td>E25</td>
<td>C6</td>
<td>1</td>
</tr>
<tr>
<td>IVS23−2A&gt;G</td>
<td>g15907A&gt;G</td>
<td>c2309−2A&gt;G</td>
<td>I23</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>IVS27+10C&gt;T*</td>
<td>g18671C&gt;T</td>
<td>c2905+10C&gt;T</td>
<td>I27</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>R1022P</td>
<td>g20021G&gt;C</td>
<td>c3065G&gt;C</td>
<td>E29</td>
<td>C8</td>
<td>1</td>
</tr>
<tr>
<td>IVS30+5G&gt;C</td>
<td>g20514G&gt;C</td>
<td>c3330+5G&gt;C</td>
<td>I30</td>
<td>–</td>
<td>1</td>
</tr>
</tbody>
</table>

*Bold* mutations not previously described. C, Ig-like domain numbered consecutively (C1, C2, C3, C5, C6, C8). *Number of patients with a mutation found in this study.

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Localization

Topological placement of the mutations was done using the Swissprot database (http://ca.expasy.org/uniprot/) and the bibliography previously described. The Uniprot database provides generally accepted residue ranges corresponding with each domain region and specialized subregion.

Predicting Damaging Amino Acid Substitution

Five online tools were used to predict the pathogenicity of the missense variants: SIFT (http://sift.jcvi.org/www/SIFT_seq_submit2.html),

Predicting Splice Site Variants


Predicting Truncation

To predict the altered reading frame in the nonsense mutation, the online software Open Reading Frame (ORF) Finder from the NCBI (www.ncbi.nlm.nih.gov/projects/gorf/) was used. For this purpose, the consensus coding sequence (CCDS) of the reference sequence and of the mutated sequence were used, and the frame used was started at NC_000011.9 (47351957…47375253); TPM1: NC_000015.9 (61121891…61151167); TNNJ3: NC_000019.9 (55662135…55670100); TNNT2: NC_000001.10 (199594765…199613428).

Classification of Genetic Variants

A variant was considered a mutation, and thus candidate for a somatic mutation, in accordance with the following criteria: absence of the mutation in 200 healthy adult controls and conservation of the mutated residue.

Moreover, the variants previously described were revised to assess their pathogenicity (Table S1) and the new mutations were studied using in silico tools.

<table>
<thead>
<tr>
<th>Table 3. Mutations Found in MYH7 Gene</th>
</tr>
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<tbody>
<tr>
<td>Mutation</td>
</tr>
<tr>
<td>A3555*</td>
</tr>
<tr>
<td>L427M*</td>
</tr>
<tr>
<td>D587H*</td>
</tr>
<tr>
<td>V606E</td>
</tr>
<tr>
<td>R719Q</td>
</tr>
<tr>
<td>A797T</td>
</tr>
<tr>
<td>R845K*</td>
</tr>
<tr>
<td>K847E</td>
</tr>
<tr>
<td>K847del</td>
</tr>
<tr>
<td>E1356K</td>
</tr>
<tr>
<td>R1382Q*</td>
</tr>
<tr>
<td>E1555G*</td>
</tr>
<tr>
<td>A1637T*</td>
</tr>
<tr>
<td>N1890S*</td>
</tr>
<tr>
<td>G1931C*</td>
</tr>
</tbody>
</table>

*Bold*, mutations not previously described. S1, globular motor domain; U50, upper 50-kDa subdomain; L50, lower 50-kDa subdomain; 20 kDa, C-terminal converter subdomain; S2, S2 domain; LMN, light meromyosin domain. *Number of patients with a mutation found in this study.

<table>
<thead>
<tr>
<th>Table 4. Mutations Found in TNNT2, TNNI3 and TPM1 Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
</tr>
<tr>
<td>R278C</td>
</tr>
<tr>
<td>R186Q</td>
</tr>
<tr>
<td>IVS5-3C&gt;T*</td>
</tr>
</tbody>
</table>

*Bold*, mutations not previously described. *Number of patients with a mutation found in this study.
Characterization of Mutations

In our cohort, we described 41 carriers with 37 different mutations, as several patients carried the same mutations (3 patients had MYBPC3 IVS17+4A>T, 2 had MYBPC3 V219L, 2 had MYH7 A797T, and 3 had MYH7 K847E) and others patients carried more than 1 mutation as we have just stated.

The mutation type has been described as a risk factor in several heart diseases, as channelopathies; however, its role in HCM is a controversial theme. The distribution of the different mutation types in our study was 64.8% missense (n=24), 29.8% splicing (n=11), 2.7% nonsense (n=1), and 2.7% deletions (n=1).

As far as we know, 17 of 37 mutations found in our cohort have not been previously described. To determine the pathogenicity of these mutations, we performed an in silico study of them according to the type of mutation.

Novel Mutations in MYBPC3

As it is shown in Table 2 and Figure 1A, 6 novel mutations were found in MYBPC3. Interestingly, all these mutations would produce truncated proteins, as they were splicing and nonsense mutations.

Results

A total of 104 samples of cardiac tissue were included in the study; the clinical characteristics of patients are summarized in the Table 1. The 43 mutations identified in 41 patients are shown in Tables 2–4. Every nucleotide sequence variant found in the tissue samples was also found in the blood samples taken from the same patient, indicating that these mutations were germline variants. Therefore, we found no evidence of MYH7, MYBPC3, TNNT2, TNNI3, and TPM1 somatic sequence variants in any of our samples.

Focusing on the distribution of the disease genes, it was shown that the most frequent genes involved in the genotyped index patients were MYBPC3 and MYH7, which were mutated in 49% and 42%, respectively, of cases of mutations. The others genes (TNNT2, TNNI3, and TPM1) were involved in approximately 10% of cases of a mutation (Table 3). Two patients carried more than 1 mutation: 1 patient had 1 mutation in MYH7 (A797T) and another in MYBPC3 (R1022P) and the other patient had 2 mutations in MYBPC3 (A216T and E258K). This gives us 1.9% of cases of multiple mutation.

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Figure 1B shows 5 web-based tools designed to provide predictions of the effect of nucleotide variations on splicing the position +1.
Figure 2. (A) Schematic structure of the human myosin-β protein showing the new mutations identified in the present study that cause hypertrophic cardiomyopathy (HCM). (B) Predictions of SIFT, Pmut, Polyphen, SNAP, and PhDSNP for the new mutations described in MYH7. The pathological predictions are shown in red.

Figure 3. (A,C) Schematic structure of the human TNNI3 and TPM1 ISO1 genes showing the new mutations identified in the present study that cause hypertrophic cardiomyopathy (HCM). (B,D) Predictions of GeneSplicer, SSF, MaxEnt, NNSPLICE, and HSF for the new mutations described in TNNI3 and TPM1 ISO1. The score of the consensus sequence vs. the mutant sequence, using each software program, is shown.
sites. As it has been used in other studies,25 the mutation score should be at least 20% lower than the wild type score in order to consider the prediction as positive and deleterious. The 5 bioinformatic tools predicted that IVS22-1G>A skipped the splicing acceptor and 4 of the 5 tools indicated that IVS7+2T>C and IVS17+5G>T altered the donor sites. However, all the tools predicted that the variants IVS11+8C>T and IVS27+10C>T would not affect the splicing process.

**Figure 1C** shows the ORF in the CCDS consensus sequence and in the W711X patient sequence in which in the 711 position a stop codon was introduced.

**Novel Mutations in MYH7**

As shown in **Table 3** and **Figure 2A**, 9 of the 15 mutations found in **MYH7** were novel. In silico analysis of the new missense mutations was performed using a suite of bioinformatic tools (**Figure 2B**). Analysis in SIFT, Pmut, PolyPhen, SNAP and PhDSNp revealed that for the E1555G mutation there was a consensus between the different bioinformatic tools to classify it as pathological. But, for 6 mutations (A355S, L427M, D587H, R1382Q, N1890S, and G1931C), at least 1 program did not predict a clear effect on protein function. Moreover, 2 of these were variants, R845K and A1637T, predicted to be neutral by all 5 methods.

**Novel Mutations in TNNI3 and TMP1**

As shown in **Table 4** and **Figure 3**, 2 new mutations were described in **TNNI3** and **TMP1**. Both of them were splicing site mutations and an in silico study, as previously described, was performed. The 5 bioinformatic tools predicted that neither of 2 variants would affect the encompassing exons process (**Figure 3**).

**Previously Described Mutations**

To confirm the pathogenicity of the 20 previously described mutations, an exhaustive review of the literature was performed (**Table S1**).

All the variants were described as pathogenic, but in 3 cases a favorable prognosis was described (**MYH7**: V606M, A797T, and K847E); 12 of the variants were associated with a family history of SCD. Moreover, other mutations associated with 7 variants found in our cohort (**MYBPC3**: E258K, R502W, IVS17+4A>T, A833T, and R1022P; **MYH7**: V606M, R719Q) have been described.

In most of the cases analyzed in the review of the literature (75%), a familial study had been performed and in the vast majority of the cases an incomplete penetrance was described. However, only in 35% was a functional study performed (**Table S1**).

Interestingly, 5 of the amino acids, where the mutations were found, were “hotspots” (**MYBPC3**: R495, R502, and A833; **MYH7**: R719; **TNNI2**: R278) and a founder effect was suggested in 3 variants (**MYBPC3**: E258K and A833T; **MYH7**: A797T).

**Discussion**

In the present study, we identified 43 mutations in DNA isolated from cardiac tissue obtained from patients with HCM, and all these variants were also found in DNA isolated from blood. The data demonstrated that all the variants were germ-line sequence variants, and none of them was a tissue-specific or a somatic mutation.

Moreover, this report describes the screening of 5 genes in a population of 104 unrelated index cases with sporadic forms of HCM. Mutations were identified in 41 index cases (39.4%), a percentage that is in concordance with data previously described.12

**Role of Somatic Mutation in Heart Disease**

For nearly a century, the general belief has been that the heart is a terminally differentiated post-mitotic organ in which the number of cardiomyocytes is established at birth, with these cells persisting throughout the lifespan of the organ and organism.35 However, nowadays is known that myocytes possess a certain degree of developmental plasticity and are able to dedifferentiate and acquire a proliferative state.36–38 Thus, somatic mutations might take place at this stage. Moreover, somatic mosaicism in cardiac tissue also could result from somatic mutation in an early myocardial progenitor cell during embryogenesis.

The first description of a somatic mutation in cardiac tissue was made by Dr. Lerman, who found the point variant F200L in the GTP binding domain of the inhibitory G protein in a biopsy sample from the arrhythmogenic focus obtained from a patient with idiopathic ventricular tachycardia.39 Moreover, this process has also been described in several cases of atrial fibrillation where somatic mutations in **GJA1** and **GJA5** that encode connexin 40 and 43, respectively, have been detected. However, somatic mosaicism in the congenital heart diseases is controversial because some studies have detected cardiactissue-specific mutations37–40 while others have failed to detect them.41,42

In the present study, no evidence of somatic **MYH7**, **MYBPC3**, **TNNI3**, and **TPM1** sequence variants was found in any of the samples described herein. Thus, other strategies must be used to determine the etiology of HCM in genotype-negative patients.

**Mutations in the Cohort**

The distribution of the disease genes of the carriers series was as follows: **MYBPC3**, 48.9%; **MYH7**, 41.9%; **TPM1**, 2.3%; **TNNI3**, 2.3%; and **TNN12**, 4.6%. These results differ from previously reported estimates in which **MYH7** was the most frequent,43,44 but in concordance with more recent reports that indicate that mutations in **MYBPC3** are the most frequent.10,12,21,41 However, we can not discount that other mutations in other sarcomeric and non-sarcomeric genes may cause HCM.

The percentage described in this study for multiple mutations (2 cases representing 1.9%) was similar to that described in the literature, at around the 3%.10,11,21,46 The minor differences could be related to polymorphic variants considered as mutations and the inclusion of related probands. In our study, we considered a variation as a mutation if it was not present in 200 unrelated healthy individuals, and our index cases were unrelated.

**Mutations Not Previously Described**

The pathogenicity of the new mutations must be established based on the criteria of absence in healthy controls, conservation in evolution and planned functional alteration. In this study, none of the previously not described mutations was present in controls, and they were conserved across more than 8 species. To study the functional alterations that the mutations can produce in the proteins, we used several in silico tools that are designed to predict the pathogenicity of each type of mutation.

Pathogenicity is very likely in mutations that provoke the truncation of the protein; in fact, this type of mutation has been associated with the most severe manifestations of HCM.43,45
our cohort, we described 6 new mutations in MYBPC3, which produced truncated proteins either because it introduced a premature stop codon (MYBPC3-W711X) or because it altered the splicing binding site (MYBPC3-IVS7+2T>C, IVS11+8G>T, IVS17+5G>T, IVS22-1G>A, IVS27+10C>T). Moreover, we described 2 intronic mutations in minority genes: 1 in TNNI3 (IVS6-12G>A) and the other in TPM1 (IVS3-3C>T). To evaluate if the point mutations in the splicing sites could alter the accuracy of intron excision and exon junction during pre-mRNA splicing, we used some web-based tools designed to provide this type of prediction. In fact, we used a combination of complementary in silico tools28–32 that run distinct algorithms, as the weaknesses of 1 in silico tool may be overcome by the results of another tool, as has been previously described.33 The result of this analysis showed that only 3 of the mutations (IVS7+2T>C, IVS17+5G>T, and IVS22-1G>A), all in MYBPC3, may have a functional effect on the protein structure, as they may modify the 5’ donor or the 3’ acceptor splice sites. However, functional studies of these mutations will be essential to elucidate whether they act through a dominant-negative mechanism,47 if the resulting protein continues to be incorporated into the A-band, or through haploinsufficiency,48 if the enhanced proteolysis of the truncated protein rather alters the stoichiometry of sarcomeric proteins.49

Pathogenicity is more difficult to confirm in new mutations of the missense type. However, an increasing number of computational approaches to in silico analysis of substitutions available on the worldwide web has been proposed to discriminate between amino acid substitutions that may affect the stability or the function of the protein, leading to a disorder, and neutral variations that do not modify the phenotype.45–50 We used this approach to analyze the pathogenicity of the not previously described missense mutations that interestingly were all detected in MYH7. We performed the analysis with a suite of different tools, because they use different approaches to perform their predictions (ie, SIFT-sequence based method, and Polyphen-structure based method). The in silico tools clearly predicted that the E1555G variant was a damaging mutation, whereas R845K and A1637T were neutral variants, as the 5 software programs made the same prediction. However, the predictions for the other variants (A355S, L427M, D587H, R1382Q, N1890S, and G1931C) were more difficult to assess, because several programs predicted them as pathologic while other programs predicted neutral. Taking into account this information, we can conclude that although bio-informatic prediction software is a useful tool, interpretation of the prediction must be done carefully, and it must be remembered that the gold standard in assigning pathogenicity to mutations is to demonstrate genetic evidence (ie, co-segregation in families) and an effect on protein function.41 Thus, the lack of functional and familial studies does not permit establishment of a cause-effect relationship between the novel variants and HCM.

Mutations Previously Described
Identification of a known mutation and review of the literature allow a more precise evaluation of the correlation between genotype and phenotype, and a more adequate interpretation of the pathogenic role of each mutation.

Two important types of studies must be evaluated: familial studies and functional studies. However, in the information reviewed in the literature about the mutations found in our cohort, although in 75% the familial studies were performed, only in 35% were functional studies performed. Thus, an important point could be the performance of more functional studies to improve knowledge of the mechanism underlying the alteration produced by the mutation and which would cause the HCM.

Moreover, other types of valuable information that can be extracted from the literature are the association with mild or severe clinical disease course, family history of SCD and the presence of more than 1 mutation responsible for HCM.

Finally, it is important to note that the description of a new mutation will generate information that can be used to assess the pathogenicity of the same mutation in other patients. Because of this, it is essential that when a mutation is published, all the relevant clinical information, familial studies, and functional studies are included.

Conclusions
The present study identified constitutional mutations in a HCM cohort, but was unable to find somatic variants. Thus, and because of the large cohort studied, other strategies must be used to determine the etiology of HCM in genotype-negative patients.

Moreover, in silico analysis must be carefully taken into account, because the consequence of an erroneous prediction may be disastrous from the perspective of genetic counseling.

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Disclosures
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