Invest Clin 55(1): 23 - 31, 2014

Genetic variations of β-*MYH7* in Venezuelan patients with hypertrophic cardiomyopathy.

Rosalva Rodríguez[1], David Guerrero[1], Yoyna Rivas[1], Andrea Lacruz[1] and Yris Flores[2].

[1]Laboratorio de Genómica de Enfermedades Musculares,
Centro de Biología Estructural, Instituto Venezolano de Investigaciones Científicas (IVIC).
Miranda, Venezuela.
[2]Asociación Cardiovascular Centro-Occidental (ASCARDIO). Barquisimeto, Venezuela.

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 β-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.

Autor de correspondencia: Rosalva Rodríguez. Laboratorio de Genómica de Enfermedades Musculares, Centro de Biología Estructural, Instituto Venezolano de Investigaciones Científicas (IVIC). Miranda, Venezuela. Teléf. 58-212-5041715. Correo electrónico: rosalvarodriguez29@gmail.com.

Variaciones genéticas en el gen *MYH7* en pacientes venezolanos con miocardiopatía hipertrófica.

Invest Clin 2014; 55(1): 23 - 31

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

Resumen. La miocardiopatía hipertrófica (MH) es una enfermedad cardiaca primaria, caracterizada por una marcada hipertrofia y variabilidad genética. MH ha sido asociada con mutaciones en las proteínas del sarcómero, siendo la beta miosina cardiaca, 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 involucraran un cambio de aminoácido en ninguno de los exones del gen *MYH7* de la beta miosina cardiaca.

Recibido: 26-07-2013. Aceptado: 13-02-2014

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

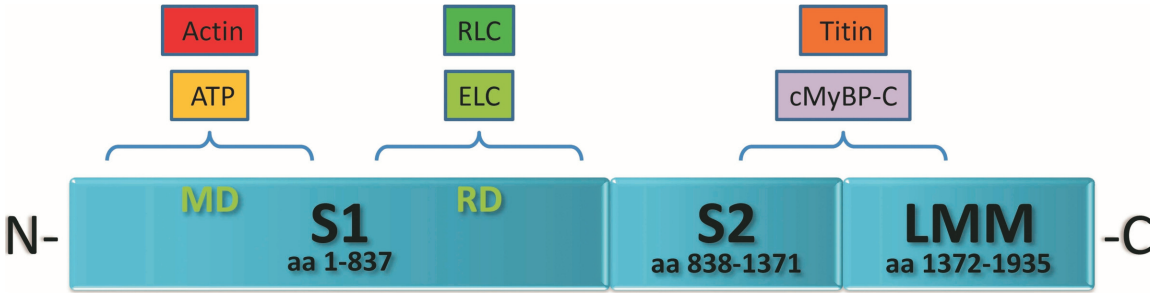

Fig. 1. Diagram of the cardiac β-miosin heavy chain coded by the *MYH7* gene. It consists of three regions: subfragment 1 (S1), subfragment 2 (S2) and light meromyosin (LMM). The S1 includes a motor and a regulatory domains (MD and RD). The motor domain includes the amino terminal, and specifically binds to actin and Mg.ATP. The regulatory domain includes also two light chains: the esential (ELC) and the regulatory light (RLC) chains. The S2 and LMM interacts with cMyBP-C and titin. The C-terminus is in the LMM.

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
CLINICAL CHARACTERISTICS OF HCM PATIENS

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

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).

TABLE II
MYH7 GENE POLYMORPHISM FOUND IN VENEZUELAN HCM PATIENTS

Exon	Gen position	Protein position	Control/Patients N=106/58	Allelic frecuency (control)	Allelic frecuency (Patients)	SNP
E3	g.5909 T>C	Thr 63 Thr	81/22	T=0.382 C=0.618	T=0.224 C=0.776	rs2069540
E12	g.9633 G>A	Lys 365 Lys	18/8	G=0.084 A=0.915	G=0.077 A=0.922	rs735711
E12	g.9666 C>T	Asp 376 Asp	20/11	C=0.099 T=0.900	C=0.112 T=0.887	rs2231126
E12	g.11573A>G	Glu 535 Glu	5/4	A=0.023 G=0.976	A=0.034 G=0.966	rs2069543
E16	g.11723 C>A	Ile 585 Ile	1/0	C=0.004 A=0.995	C=0 A=1	rs201860580
E16	g.11735 C>T	Asn 589 Asn	0/3	C=0 T=1	C=0.025 T=0.974	rs3729816
E16	g.15354 T>C	Ala 917 Ala	106/29	T=0.5 C=0.5	T=0.25 C=0.75	rs1041957
E23	g.15430 T>C	Leu 943 Leu	106/29	T=0.5 C=0.5	T=0.25 C=0.75	rs2856898

SNP: Single nucleotide polymorphism.

TABLE III
MYH7 GENE INTRONIC VARIATIONS FOUND IN VENEZUELAN HCM PATIENTS

Position in the gene	Intron	Change in the nucleotide sequence	Control/Patients N= 106/58	Allelic Frequency (Controls)	Allelic Frequency (Patients)
g.12721	IVS15-256	C>T	3/0	C=0.014 T=0.985	C= 0 T=1
g.14788	IVS19-17	A>G	55/20	A=0.259 G=0.741	A=0.172 G=0.827

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

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.

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 previously classified with specific heart pathologies, as it is done in a large reference center; second, the patient population in the study could represent sporadic cases of HCM. Other studies have noted that in sporadic cases of HCM there can be a scarcity of mutations in the genes most commonly mutated in HCM patients (28). Also, it has been demonstrated that the frequency of mutations in the *MYH7* gene is low in patients diagnosed as mature adults (11, 18), which could explain the absence of mutations in the 55 years old patients in our study . According to Brito *et al.* (28) individuals with sporadic HCM exhibit the disease later in life and the causative mutations often remained unidentified in familial hypertrophic cardiomyopathy (FHC) cases.

The number of mutations associated with FHC is dependent upon the genetic characteristics of the population studied. In one study by Laredo *et al.* in Spain (18), mutations in the *MYH7* gene were reported only in 10% to the families evaluated. Similar results were observed by Van driest *et al.* (29) and Garcia Castro *et al.* (30).

Additionally, Roncaratti *et al.* analyzed a population of 125 unrelated Italian patients and reported a low number of mutations in the *MYH7* gene (31). Most studies point to an association between mutations in the *MYH7* gene and familial history of HCM, but this was not found in the study of Bashyam *et al.* (32), which reported mutations in only seven of 80 patients, with familial history, suggesting that the role of the mutations in the *MYH7* gene in FHC is not completed defined and may depend upon the genetic heterogeneity of the populations studied.

The clinical variability observed in HCM disease, could be influenced by several factors, such as modifier genes, epigenetic factors, microRNAs, posttranslation protein modifications and environmental factors (1,

24, 25). The clinical heterogeneity is demonstrated by instances where two individuals from the same family share the same mutation but exhibit different sympthomatology; 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.

REFERENCES

1. **Millat G, Bouvagnet P, Chevalier P, Dauphin C, Jouk PS, Da Costa A, Prieur F, Bresson JL, Faivre L, Eicher JC, Chassaing N, Crehalet H, Porcher R, Rodriguez-Lafrasse C, Rousson R.** Prevalence and spectrum of mutations in a cohort of 192 unrelated patients with hypertrophic cardiomyopathy. Eur J Med Genet 2010; 53(5):261-267.
2. **Zou Y, Wang J, Liu X, Wang Y, Chen Y, Sun K, Gao S, Zhang C, Wang Z, Zhang Y, Feng X, Song Y, Wu Y, Zhang H, Jia L, Wang H, Wang D, Yan C, Lu M, Zhou X, Song L, Hui R.** Multiple gene mutations, not the type of mutation, are the modifier of left ventricle hypertrophy in patients with hypertrophic cardiomyopathy. Mol Biol Rep 2013; 40(6): 3969-3976.
3. **Frey N, Luedde M, Katus HA.** Mechanisms of disease: hypertrophic cardiomyopathy. Nat Rev Cardiol 2011; 9(2): 91-100.
4. **Marian AJ.** Hypertrophic cardiomyopathy: from genetics to treatment. Eur J Clin Invest 2010; 40(4): 360-369.
5. **Walsh R, Rutland C, Thomas R, Loughna S.** Cardiomyopathy: a systematic review of disease-causing mutations in myosin heavy chain 7 and their phenotypic manifestations. Cardiology 2010; 115(1): 49-60.
6. **Seggewiss H, Blank C, Pfeiffer B, Rigopoulos A.** Hypertrophic cardiomyopathy as a cause of sudden death. Herz 2009; 34(4): 305-314.
7. **Harris SP, Lyons RG, Bezold KL.** In the thick of it: HCM-causing mutations in myosin binding proteins of the thick filament. Circ Res 2011; 108(6): 751-764.
8. **Ho CY.** New Paradigms in Hypertrophic Cardiomyopathy: insights from genetics. Prog Pediatr Cardiol 2011; 31(2): 93-98.
9. **Maron BJ, Maron MS, Wigle ED, Braunwald E.** The 50-year history, controversy, and clinical implications of left ventricular outflow tract obstruction in hypertrophic cardiomyopathy from idiopathic hypertrophic subaortic stenosis to hypertrophic cardiomyopathy. J Am Coll Cardiol 2009; 54 (3): 191-200.
10. **Maron BJ, Maron MS, Semsarian C.** Genetics of hypertrophic cardiomyopathy after 20 years: clinical perspectives. J Am Coll Cardiol 2012; 60(8): 705-715.
11. **Tian T, Liu Y, Zhou X, Song L.** Progress in the molecular genetics of hypertrophic cardiomyopathy: a mini-review Gerontology 2013; 59(3): 199-205.
12. **Marian AJ.** Genetic determinants of cardiac hypertrophy. Curr Opin Cardiol 2008; 23(3): 199-205.
13. **Konno T, Chang S, Seidman JG, Seidman CE.** Genetics of hypertrophic cardiomyopathy. Curr Opin Cardiol 2010; 25(3): 205-209.
14. **Zheng DD, Yang JH, Tao Q, Geng M, Lin J, Yang XJ, Song JP, Li HX, Han LH,**

Jiang WP. Mutations in the beta-myosin heavy chain gene in southern Chinese families with hypertrophic cardiomyopathy. J Int Med Res 2010; 38(3): 810-820.

15. **Ackermann MA, Kontrogianni-Konstantopoulos A.** Myosin binding protein-C: a regulator of actomyosin interaction in striated muscle. J Biomed Biotechnol 2011; 636403-636412.
16. **Tajsharghi H, Oldfors A.** Myosinopathies: pathology and mechanisms. Acta Neuropathol 2013; 125(1): 3-18.
17. **Maron Barry J, Maron Martin S.** Hypertrophic cardiomyopathy. The Lancet 2013; 381(9862): 242-255.
18. **Laredo R, Monserrat L, Hermida-Prieto M, Fernández X, Rodríguez I, Cazón L, Alvariño I, Dumont C, Piñón P, Peteiro J, Bouzas B, Castro-Beiras A.** Beta-myosin heavy-chain gene mutations in patients with hypertrophic cardiomyopathy. Rev Esp Cardiol 2006; 59(10): 1008-1018.
19. **Lahiri DK, Nurnberger J**. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 1991; 19: 5444.
20. **Villard E, Duboscq-Bidot L, Charron P, Benaiche A, Conraads V, Sylvius N, Komajda M.** Mutation screening in dilated cardiomyopathy: prominent role of the beta myosin heavy chain gene. Eur Heart J 2005; 26(8): 794-803.
21. **Daehmlow S, Erdmann J, Knueppel T, Gille C, Froemmel C, Hummel M, Hetzer R, Regitz-Zagrosek V.** Novel mutations in sarcomeric protein genes in dilated cardiomyopathy. Biochem Biophys Res Comm 2002; 298(1): 116-120.
22. **Erdmann J, Daehmlow S, Wischke S, Senyuva M, Werner U, Raible J, Tanis N, Dyachenko S, Hummel M, Hetzer R, Regitz-Zagrosek V.** Mutation spectrum in a large cohort of unrelated consecutive patients with hypertrophic cardiomyopathy. Clin Genet 2003: 64(4): 339-349.
23. **Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vosberg HP, McKenna W, Seidman CE, Seidman JG.** A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. Cell 1990; 62(5): 999-1006.
24. **Maron BJ, Maron MS.** Hypertrophic cardiomyopathy. Lancet (2013); 381 (9862): 242-255.
25. **Xu Q, Dewey S, Nguyen S, Gomes AV.** Malignant and benign mutations in familial cardiomyopathies: insights into mutations linked to complex cardiovascular phenotypes. J Mol Cell Cardiol 2010; 48(5): 899-909.
26. **Armel TZ, Leinwand LA.** Mutations in the beta-myosin rod cause myosin storage myopathy via multiple mechanisms. Proc Natl Acad Sci USA 2009; 106(15): 6291-6296.
27. **Blair E, Redwood C, de Jesus Oliveira M, Moolman-Smook JC, Brink P, Corfield VA, Ostman-Smith I, Watkins H.** Mutations of the light meromyosin domain of the beta-myosin heavy chain rod in hypertrophic cardiomyopathy. Circ Res 2002; 90(3): 263-269.
28. **Brito D, Richard P, Komajda M, Madeira H.** Familial and sporadic hypertrophic myopathy: differences and similarities in a genotyped population. A long follow-up study. Rev Port Cardiol 2008; 27(2):147-173.
29. **Van Driest SL, Ommen SR, Tajik AJ, Gersh BJ, Ackerman MJ.** Sarcomeric genotyping in hypertrophic cardiomyopathy. Mayo Clin Proc 2005; 80(4):463-469.
30. **Garcia-Castro M, Coto E, Reguero JR, Berrazueta JR, Alvarez V, Alonso B, Sainz R, Martin M, Morris C.** Mutations in sarcomeric genes MYH7, MYBPC3, TNNT2, TNNI3, and TPM1 in patients with hypertrophic cardiomyopathy. Rev Esp Cardiol 2009; 62: 48-56.
31. **Roncarati R, Latronico MV, Musumeci B, Aurino S, Torella A, Bang ML, Jotti GS, Puca AA, Volpe M, Nigro V, Autore C, Condorelli G.** Unexpectedly low mutation rates in beta-myosin heavy chain and cardiac myosin binding protein genes in Italian patients with hypertrophic cardiomyopathy. J Cell Physiol 2011; 226(11):2894-2900.

32. **Bashyam MD, Purushotham G, Chaudhary AK, Rao KM, Acharya V, Mohammad TA, Nagarajaram HA, Hariram V, Narasimhan C.** A low prevalence of *MYH7/MYBPC3* mutations among familial hypertrophic cardiomyopathy patients in India. Mol Cell Biochem 2012; 360(1-2): 373-382.
33. **Golbus JR, Puckelwartz MJ, Fahrenbach JP, Dellefave-Castillo LM, Wolfgeher D, McNally EM.** Population-based variation in cardiomyopathy genes. Circ Cardiovasc Genet 2012; 5(4): 391-399.