

## Investigation of Mutations in Exons 12-15 MYH7 Gene in Hypertrophic Cardiomyopathy Patients Using PCR-SSCP Technique

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### Abstract

**Background:** Hypertrophic cardiomyopathy (HCM) is the most common kind of Mendelian inherited heart disease, affects 0.2% of the global population. HCM is also the most common cause of sudden cardiac death in individuals younger than 35 years old. To date more than 900 individual mutations has been identified in over 20 genes, such as MYH7, MYBPC3, and TNNT2. Interestingly, most of these genes encode sarcomeric proteins. In the present study, we investigated the possible presence of mutation in exons 12-15 MYH7 gene, which has already been reported to accommodate some mutations, in 30 patients with HCM in Chaharmahal va Bakhtiari province.

**Materials and Methods:** DNA was extracted using standard phenol-chloroform method and then was used for amplification and gel electrophoresis by PCR-SSCP procedure. Finally, the suspected cases were selected for the direct sequencing and the results were analyzed using chromas software.

**Results:** There is no mutation in these exons, but two polymorphisms including: 5811 C>T and 5845 G> were found in the exon 12 of 1 and 5 separate patients, respectively.

**Conclusion:** In this study with respect to none amino acid codon changes arisen from these polymorphisms, we concluded that mutations in these exons of MYH7 gene have a very low contribution in patients in this province and this is necessary to study other exons for better assessment.

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### Introduction

Hypertrophic cardiomyopathy (HCM) is a heart disease of genetic origin characterized by sarcomere disarray, myofibril clutter, and interventricular septal hypertrophy and is associated with decreased left ventricular volume [1]. Other characteristics of the disease are diastolic dysfunction, myocardial ischemia, sudden cardiac death and irregular heart rate. It can be seen in all age groups, from birth to 80s. It is the most common cause of sudden death in young people and affects one in 500 of general population [2]. Genetic analysis in pre-clinical course facilitates the disease diagnosis. The risk classification of the disease can be done based on molecular criteria.

The disease may manifest in two forms; primary and secondary. The primary form of the disease is caused by factors such as hypertension and aortic stenosis, while the secondary form is due to factors such as exercise and consumption of some medications [3, 4]. The disease has a high phenotypic heterogeneity and its clinical characteristics range from severe hypertrophy with sudden cardiac death to benign form seen in heterozygote individuals. Arrhythmia and sudden cardiac death in the absence of echocardiographic sings and left ventricular

hypertrophy in patients with pathogenic genotype, represents incomplete penetrance of phenotype [5-7]. The phenotype may change with age. Hypertrophy may be characterized at birth as a severe obstructive form leading to rapid progression toward heart failure and high mortality rate [8]. In adolescents and young adults, severe left ventricular hypertrophy is associated with outflow obstruction, decreased ventricular volume, and progression toward sudden cardiac death [9, 10]. Total annual mortality rate in referral centers is reported between 2-6% [7, 9]. The disease is transmitted in an autosomal dominant manner, but only 50% of cases have a family basis [11].

Several genes which encode sarcomeric proteins including MYH7, MYBPC3, and TNNT2 genes are involved in the development of HCM. To date, about 900 mutations have been identified in 20 genes related to this disease, of them about 35% are associated with MYH7 gene. MYH7 is a large gene of about 25 kb and 40 exons that encodes  $\beta$ -myosin heavy chain, expressed predominantly in the ventricles. The results of the studies conducted on these genes suggest that most mutations resulting in HCM occur in MYH7 gene [7].

The thick filaments have  $\beta$ -myosin heavy chain which is associated with 4 light chain molecules of myosin. Heavy chain of  $\beta$ -myosin makes up 30% of cardiac myosin and 95% of ventricular myosin [12]. This protein consists of 1934 amino acids, and about 194 mutations and 15 polymorphisms have been reported in its encoding gene [13]. Complete sequencing of the gene in HCM patients showed that most mutations were located in exons 8-24 which encode the globular head and head and tail links. Given that few studies were performed in Iran on the molecular basis of the disease, such as the study of Montazeri et al. on hot spots mutations of MYH7 gene [14] and mutations relevant to mitochondrial DNA and their role in HCM [15] in Tehran, we decided to study the hot spots mutations of this gene in another group of available Iranian population. The aim of this project is to examine the exons 12-15, in which some mutations have been previously reported; a total of 4 polymorphisms and 39 mutations, including a significant amount of MYH7 gene alterations. In general, MYH7 gene mutations are mainly characterized by severe myocardial hypertrophy and high penetration [13, 16-19].

## Materials and Methods

In this descriptive in vitro study, 30 patients with hypertrophic cardiomyopathy were selected from patients referring to cardiac clinic of Shahr-e-Kord University of Medical Sciences, based on clinical and echocardiographic findings [19]. Regarding to echocardiographic findings, those patients with a left ventricular wall thickness of at least 13 mm were included in the study, some of them had a family history of the disease and some had not (Table 1). After preparing the questionnaire and obtaining the written consent from patients with HCM, required blood samples were collected in tubes containing half molar EDTA and sent to the Laboratory of Cellular and Molecular Research Center and stored in  $-20^{\circ}\text{C}$  in the freezer until molecular testing. The DNA of samples were extracted through conventional phenol-chloroform method and the DNA concentration was measured spectrophotometrically (unc1 2100 USA) [20, 21]. Then, according to the MYH7 gene sequence and using Primer 3 software, the sequence of forward (F) and reverse (R) primers for exons 12 to 15 of the gene were designed and purchased (Table 2).

Each PCR microtube contained 0.3  $\mu\text{l}$  of any two F and R primers (10 ppm), 0.1  $\mu\text{l}$  of Taq polymerase enzyme (5 unit/ $\mu\text{l}$ ), 0.5  $\mu\text{l}$  of deoxynucleotides triphosphate mixture (10 mM), 2.5  $\mu\text{l}$  PCR buffer (10 X), 2  $\mu\text{l}$   $\text{MgCl}_2$  (50 mM), and 1  $\mu\text{l}$  DNA (100 ng); the final volume was reached to 25  $\mu\text{l}$  with distilled water. DNA amplification was performed in a thermal cycler (ASTEC, PC818 Japan) for 30 to 37 cycles, each thermal cycle consists of  $95^{\circ}\text{C}$  for

DNA denaturation,  $56-67^{\circ}\text{C}$  for annealing of the primers to the target DNA, and  $72^{\circ}\text{C}$  for extension of complementary strands (Table 3). Single-Strand Conformation Polymorphism (SSCP) is based on that single-stranded DNA can obtain various conformational states, regarding its basic combination, thus, if a mutation happens in the normal DNA and leads to a change in its single-stranded spatial arrangement then its movement will be different on polyacrylamide gel [22]. Therefore, the possible mutations were tested by PCR-SSCP method that includes SSCP of PCR products and heteroduplex (HA) analysis simultaneously, as follows:

For this purpose, after amplification of desired fragments by PCR, the considered samples were electrophorized on 8% polyacrylamide gels (Merck, Germany), with 50 mA current for 1 h, which then were stained with silver nitrate to see the bands. In case of bands accuracy, the relevant PCR products were analyzed through SSCP. For this purpose, 8  $\mu\text{l}$  of each PCR product was mixed with 6  $\mu\text{l}$  of SSCP Dye and heated at  $96^{\circ}\text{C}$  for 15 minutes to completely separate the two DNA strands. Then the samples were immediately placed on ice to prevent the formation of double-stranded DNA. Also, 2  $\mu\text{l}$  of each PCR product was mixed with 3  $\mu\text{l}$  EDTA (0.5 M) and reached to  $96^{\circ}\text{C}$  in thermal cycler for 5 min, and then reduced to  $37^{\circ}\text{C}$  during 60 cycles of 30 seconds.

After electrophoresis, the polyacrylamide gels were stained with silver nitrate and the bands of DNA were revealed and analyzed. Finally, all samples suspected to have MYH7 mutations because of having different patterns on polyacrylamide gel, were sequenced for confirmation and the results of DNA sequencing were analyzed with Chromas software.

**Table 1.** Main characteristics of 30 studied patients with HCM

Male/Female	15/15 s
Mean age at diagnosis	49 (17-80) years
Left ventricle thickness	
13-16 mm	5 patients
17-19 mm	7 patients
>19 mm	18 patients
With family history of HCM	16 patients
Without family history of HCM	14 patients

**Table 2.** Designed primers and fragment length of each exon in PCR

Exon	Primers	Fragment length (bp)
12	F: aatatggggcctccctacag R: acatggccctccatgactt	210
13	F: ctcccaaagtgtctgggatta R: ctgccacccattatcatct	244
14	F: ccctgctcaatatgggtctc R: gggagcagtgagtgattgt	233
15	F: tgagcagctctgcatcaact R: ttcagggtgtaaggccaaag	247

**Table 3.** Heating program for denaturation, annealing, and extension of each exon in PCR

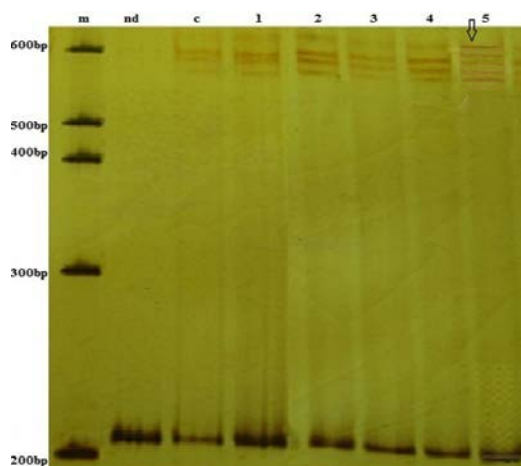
Exon	Denaturation temperature ( $^{\circ}\text{C}$ )	Duration (min)	Annealing temperature ( $^{\circ}\text{C}$ )	Duration (min)	Extension temperature ( $^{\circ}\text{C}$ )	Duration
12	95	1	56-57	1	72	1 min
13	95	1	60-61	1	72	50 sec
14	95	1	67	1	72	50 sec
15	95	1	67	1	72	50 sec

## Results

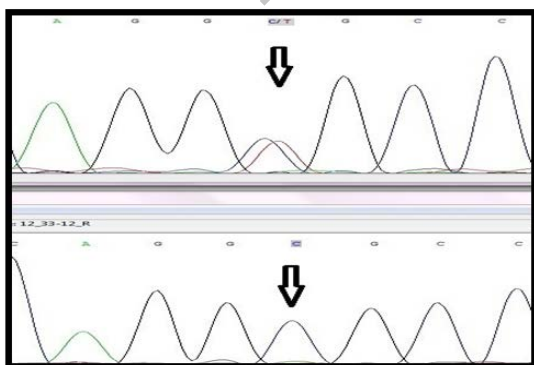
After amplification of DNA fragments isolated from blood samples of 30 patients using PCR and SSCP procedures, 6 suspected samples with a change in exon 12 were observed; those showing different banding pattern on gel compared to remaining samples which showed no change (Fig. 1, 3). However, for other exons the results of PCR-SSCP and heteroduplex analysis did not show any changes. This shows exons 13 and 14 and 15 may have the least contribution in this population.

Finally for confirmation, these 6 suspected samples were sequenced. The results of sequencing showed two silent polymorphisms, one as C>T 5811 in a 60-year-old woman with family history of the disease and the other as G>A 5845 in 5 patients including a 60-year-old man, a 21-year-old woman, and a 65-year-old man with family history of the disease, and two women of 75 and 81 years old without family history of the disease (Fig. 2, 4).

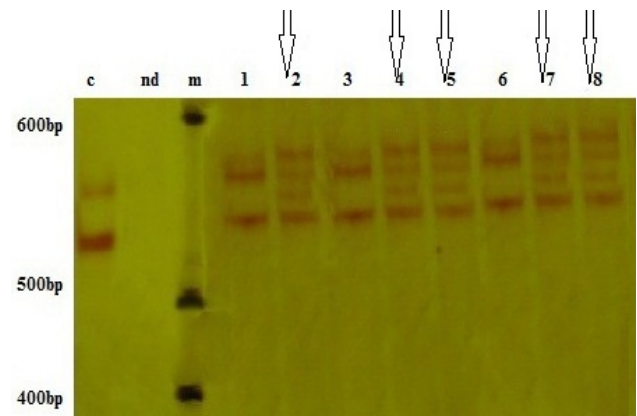
PCR-SSCP has been conventionally used as a screening method for mutations, it is relatively sensitive and accurate [22, 23]. In this study, simultaneous use of PCR-SSCP and HA had provided a high accuracy in detection of MYH7 gene mutations because of the convergence between the results of PCR-SSCP and HA which verified with DNA sequencing method.



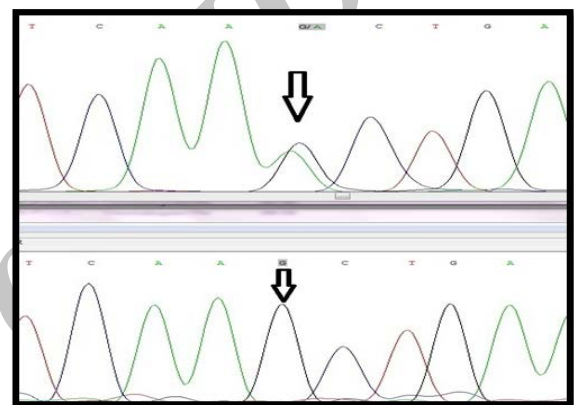
**Figure 1.** C>T 5811 polymorphism. m: marker, nd: non-denatured control, c: healthy control, 1 to 5: patients. The pattern of patient 5 differs with others and has an additional band.



**Figure 2.** Sequencing result of exon 12 of MYH7 gene, C>T 5811 polymorphism. The above chromatogram belongs to a heterozygous patient and the below chromatogram belongs to a healthy individual.



**Figure 3.** G>A 5845 polymorphism. c: healthy control, nd: non-denatured control, m: marker, 1 to 8: patients. The pattern of patients 2, 4, 5, 7, and 8 differ with others and have an additional band



**Figure 4.** Sequencing result of exon 12 of MYH7 gene, G>A 5845 polymorphism. The above chromatogram belongs to a heterozygous patient and the below chromatogram belongs to a healthy individual.

## Discussion

Hypertrophic cardiomyopathy is mainly caused by mutations in one of the sarcomeric genes. To date, about 194 mutations have been identified in humans, of them 115 mutations are in MYH7 gene [24]. Several studies have been conducted on the role of MYH7 mutations in the development of hypertrophic cardiomyopathy, such as the study of Liu et al. to screen the mutations of genes responsible for the disorder in 10 pedigrees with hypertrophic cardiomyopathy in Chinese population. In this study, the functional regions of MYH7 gene were screened by PCR and direct sequencing techniques. MYH7 mutations were found in 3 pedigrees, 3 patients were suddenly died at age 20-48 years during exercise. The results of this study showed that mutations of MYH7 in HCM patients are associated with high risk of sudden cardiac death [25].

Wang et al. (2008) had collected blood samples of two families with HCM and 120 healthy people and studied mutations of MYH7, MYBPC3, and TNNT2 genes using PCR and found most mutations occurred in MYH7 gene [19].



Garcia et al. sequenced exons 13-16 of MYH7 gene in 30 patients with HCM, in age range of 18-60 years, in Australia, of them 25 patients had a family history. In this study it was observed that one proband had nucleotide change, including a mutation (Arg 453 Cys, exon 14) in MYH7 gene [26]. Villard et al. analyzed all exons of the two genes MYH7 and TNNT2 using SSCP in 96 patients (54 patients with a family history and 42 sporadic patients). MYH7 gene mutation was observed in 5 patients with a family history and in 2 sporadic patients in head and tail of the protein. In this study it was found that most mutations were located in the MYH7 gene and also the disease onset was delayed [27]. Garcia et al. studied the frequency of sarcomere genes in 120 patients with HCM in Spain. Of them, 16% had a family history. Five genes were examined in this study and mutations were found in 32 patients, of them 10 mutations were in MYH7 gene [27].

In Iran, Montazeri and his co-workers studied hot spots mutations of MYH7 gene, including exons 13-15 in 50 patients and reported a G10195A mutation in exon 13 and a A10419C mutation in exon 14 [14]. The results of few studies in Iran and extensive studies in other parts of the world on this gene and its effect on hypertrophic cardiomyopathy suggest that exons 8-24 of the MYH7 gene are most affected in the disease. These exons encode the globular head and neck of myosin and are highly susceptible to acquire a mutation. In the present study exons 12-15 of the MYH7 gene were examined, which were among the gene's hot spots and some mutations have been previously reported in them, including two mutations and a polymorphism in exon 12, twelve mutations and a polymorphism in exon 13, eleven mutations and a polymorphism in exon 14, and fourteen mutations and a polymorphism in exon 15, that consists the most changes in MYH7 gene.

No changes were found in exons 13 to 15, although the results of this study revealed two silent polymorphisms including C>T 5811 and G>A 5845 in exon 12 of the MYH7 gene. These silent polymorphisms have been previously reported and their reference numbers are rs735712 and rs735711, respectively [28]. In fact, these two polymorphisms do not make any change in the

sequence of amino acids of beta myosin; the first polymorphism is C>T 5811 causes a change of GGC codon to GGT, both encoding glycine, and the second polymorphism is G>A 5845 leads to a change of AAA codon to AAG, both encoding lysine. Based on this study, mutations in the exons 13 to 15 of MYH7 gene and its role in hypertrophic cardiomyopathy is minor in the population of this province and it seems necessary to perform more research on other exons and on larger samples to understand the more precise role of different exons of the gene in the disease.

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### Authors' Contributions

The introduction of HCM patients were conducted by Dr A. Khaledifar, a heart specialist and director of the project. The genetics work was conducted under supervision of Dr R. Pourahmad as a supervisor and Dr. M. Hashemzadeh as an advisor and the head of Cellular and Molecular Research Center both are geneticists. Miss S. Hidari is a M. Sc student of Genetics and the major co-workers. Z. Amini and N. Khosravi are M. Sc students working in Cellular and Molecular Research Center. S. Badfar works in echocardiography department of Hajar Hospital.

### Conflict of Interest

The authors declare no conflict of interest.

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