Beta-myosin heavy-chain mutations R403QLW, V606M, K615N and R663H in patients with hypertrophic cardiomyopathy

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Abstract

Objective: Hypertrophic cardiomyopathy (HCM) is a disease of the myocardium with an autosomal-dominant pattern of inheritance mainly caused by single heterozygous mutations in sarcomere genes. In this study we aimed to detect the presence of R403QLW, V606M, K615N, and R663H mutations in beta-myosin heavy-chain gene (MYH7) and figure out the genotype-phenotype correlations in Turkish patients with HCM.

Methods: This case-control study based on genotype-phenotype correlation included 69 patients (mean age, years: 50±13.16) diagnosed with HCM constituting the study group and 50 healthy individuals (mean age, years: 52±1.4) constituting the control group. DNA was extracted from peripheral blood and the genotyping of mutations was performed by real-time PCR technique and high resolution melting analysis. Associations between categoric variables were determined using chi-square tests. Differences between two groups were compared with unpaired Student's t-test for continuous variables.

Results: None of the patients in the HCM group were carrying the index mutations. One healthy individual was found to be heterozygous for the R663H mutation with mildly abnormal IVS and LVPW thickness. The allele frequency for R663H (G>A) mutation was found to be 0.01% in control group.

Conclusion: We performed a mutational screening of 6 HCM-associated mutations in 69 Turkish HCM patients (not previously studied except R403Q). There was no significant difference in the prevalence of the mutations between the patients with HCM and the healthy controls (p>0.05). *(Anadolu Kardiyol Derg 2014; 14: 244-50)*

Key words: hypertrophic cardiomyopathy, mutation, cardiac beta-myosin heavy chain

Introduction

Hypertrophic cardiomyopathy (HCM) is a disease of the myocardium which manifests as left ventricular (LV) hypertrophy without obvious cause, with myocyte disarray and interstitial fibrosis (1) and is often associated with arrhythmias, syncope, heart failure or sudden cardiac death even at a young age (2). Clinical presentation can vary from asymptomatic to sudden death (3, 4).

HCM is a familial disease in at least 55% of cases, with an autosomal dominant mode of inheritance with variable expressivity and age-related penetrance (5, 6). Prevalence of phenotypically expressed HCM in the adult general population is about 0.2% (1, 7). HCM is caused by over 1000 different mutations identified in over 29 genes (8). Over 1400 HCM-causing or HCM-associated variants have been identified in 11 or more different genes each encoding proteins of the cardiac sarcomere with no specific racial or ethnic predilections (9). Genes which have been associated with HCM are summarized in Table 1 and updated data are available at http://cardiogenomics.med.harvard.edu.

Myosin is a hexameric motor protein that consists of 2 heavy chain subunits (MHC), 2 alkali light chain subunits (MLC) and 2 regulatory light chain subunits (MLC-2) (10) Human cardiac beta-myosin heavy chain (MHC) is 22,883 bp long and the 1935 amino acids of this protein are encoded by 38 exons (11) Mutations in the MYH7 gene are common cause of familial hypertrophic cardiomyopathy. Various studies have reported that the missense mutations in the beta-myosin heavy chain gene (MYH7) cause ~30-40% of all cases HCM (12-14). Almost all of the HCM causing missense mutations in MYH7 gene located in the globular head of myosin (subfragment 1, S1) or in the head-rod junction of the myosin molecule (Fig. 1). The head

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region contains all the necessary elements to generate movement of actin during ATP hydrolysis (15, 16). Mutations located in actin-myosin binding site or near this site could affect the actin-myosin interactions by changing MHC sequence (17, 18). It is still unclear how a missense mutation leads to cardiac hypertrophy, myocyte disarray, interstitial fibrosis or fatal arrhythmia although it may be plausible that these symptoms could occur in response to myosin dysfunction (19). The mutations investigated in this study located at a highly conserved positions (20, 21) suggesting the importance of these residues for myosin motor function and kinetics (19, 20, 22). The purpose of this study was to investigate the frequencies and phenotypic expressions of R403QLW, V606M, K615N, and R663H mutations in beta-myosin heavy-chain gene (MYH7) that could affect myosin function in patients with HCM (Fig. 2).

Methods

Study design

The study protocol was approved by the Ege University Faculty of Medicine, Ethic Committee and all participants gave written informed consent to be included in the study. Study population consisted of 69 (mean age, years: 50±13.16) Turkish patients diagnosed with HCM between 2007 and 2011. Complete physical examination and detailed clinical history including family history of HCM, syncope or sudden death in first degree relatives at a young age (<40 years), symptoms and New York Heart Association (NYHA) functional class were obtained from all the participants. Fifty healthy (mean age, years: 52±1.4) age and gender matched individuals served as the control group. Ten of the controls were from the volunteered medical staff and the rest of them consisted of subjects with sinus rhythm who had been referred to our echocardiography laboratory for the investigation of patent foramen ovale, atrial septal defect, and interatrial aneurysm. All control subjects had normal transthoracic echocardiograms.

Table 1. Sarcomere mutations in hypertrophic cardiomyopathy

Protein	Gene	Chromosome	Prevalence
Cardiac β-myosin heavy chain	*MYH7*	14q12	~40%
Cardiac myosin binding protein C	*MYBPC3*	11p11.2	~40%
Cardiac troponin T	*TNNT2*	1q32	~5%
Cardiac troponin I	*TNNI3*	19q13.4	~5%
α-Tropomyosin	*TPM1*	15q22.1	~2%
Myosin regulatory light chain 2	*MYL2*	12q24.11	~1%
Myosin, light chain 3	*MYL3*	3p21.3-p21.2	~1%
Actin	*ACTC1*	15q14	~1%
Titin	*TTN*	2q31	Rare
Myozenin	*MYOZ2*	4q26-q27	Rare
α-Myosin heavy chain	*MYH6*	14 28.01 cM	Rare

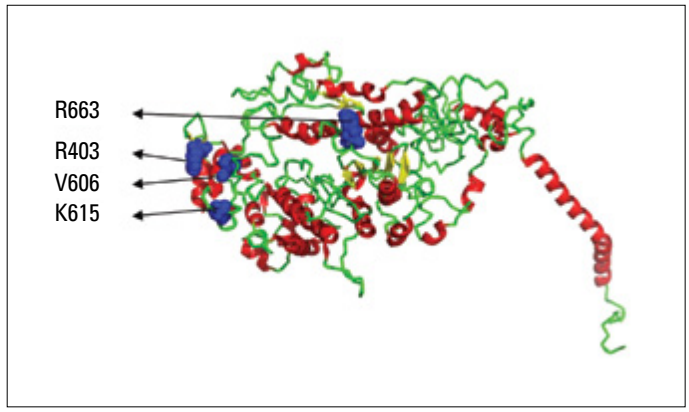

Figure 1. The 3-D diagram of human cardiac beta-myosin heavy chain. The locations of the investigated missense mutations are shown in blue. (Protein Data Bank, PDB ID:1IK2)

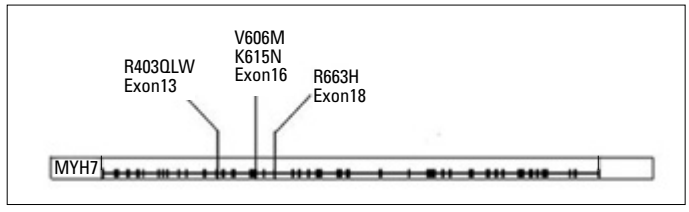

Figure 2. Location and identity of missense mutations that were investigated in this study. A schematic diagram of the normal β cardiac myosin heavy-chain gene is shown in the center (5'to 3'), and the locations of the missense mutations are shown according to exon

Echocardiographic evaluation

Transthoracic echocardiographic (SONOS 7500, Philips, Andover, MA, USA) examinations were performed according to the recommendations of American Society of Echocardiography (23). All echocardiographic examinations were recorded on VHS videotape and analyzed at study completion by 2 independent experienced physicians who were blinded with respect to patients' clinical characteristics. Standard echocardiographic analysis included M-mode, two dimensional and Doppler flow measurements.

Diagnosis

The diagnosis of HCM was based on two-dimensional echocardiographic demonstration of a hypertrophied and non-dilated LV wall thickness ≥15 mm in the absence of any cardiac or systemic disease capable of inducing LV hypertrophy (2). Left ventricle outflow tract obstruction was considered present when the peak instantaneous outflow gradient estimated by continuous wave Doppler was ≥30 mm Hg under resting conditions.

Sample preparation

Genomic DNA isolation was performed on Magna Pure LC instrument using the MagNA Pure LC DNA Isolation Kit-Large Volume according to the manufacturer's instructions (Roche Applied Science, Germany). The suitability for Real time PCR of genomic DNA was verified spectrophotometrically (NanoDrop ND-1000 Spectrophotometer, Thermo Fisher Scientific, USA). DNA samples were stored at -20°C.

Table 2. Real Time PCR primer sets, amplicon lengths and prob sets

Mutation	Primer sets	Amplicon length
R403QLW	Forward Primer:	192 bp.
	5′-TCATCTCTTTACCAACTTTGCTA-3′	
	Reverse Primer:	
	5′-CTGCCCACCCATTATCA-3′	
V606M	Forward Primer:	286 bp.
	5′-CCAGAAGCCACGCAATAT-3	
K615N	Reverse Primer:	
	5′-GAAGTGGTGGGGTGTAGC-3′	
R663H	Forward Primer:	209 bp.
	5′- CATCTCTGTGACTTCTCGAA- 3′	
	Reverse Primer:	
	5′-ACCTGGAGACTTTGTCTCA-3	
Mutation	**Probe sets**	
R403QLW	FL Prob : 5′-TGCCACCCTCAGGTGAAAGT-FL	
	LC Prob:5′-LC640-GGCAATGAGTACGTCACCAAGGGGC-PH	
V606M	FL Prob : 5′- -ACAAGCCCACGACAGTCTCA-FL	
	LCProb:5′-LC640-TGAGAGGATCCTTGTTCTTCTGCAGCCA-PH	
K615N	FL Prob: 5′- -GCCCCAGCATAGTTGGCAAACAGGG-FL	
	LCProb: 5′-LC705-GCTGAGCACCTTGAGGGAAGACT-PH	
R663H	FL Prob: 5′-CCAACTTgCACTCCACCC-FL	
	LC Prob: 5′-LC640-TCCCCACTTTGTACGTTGTATCATCCCT-PH	

Real time PCR and HRM

We performed a mutational screening of six HCM-associated mutations [R403Q (G>A), R403L (G>T), R403W (C>T), V606M (G>A), K615N (G>C), R663H (G>A)] using Real Time PCR technology in 69 Turkish patients with HCM. PCR amplification and high-resolution melting (HRM) analysis were performed using the LightCycler 2.0 instrument (Roche Diagnostics, Germany). Primer and Light Cycler probe sets were designed separately for each mutation locus in MYH7 gene and purchased from TIB MOLBIOL, Germany (Table 2). Amplicon lengths were kept relatively short to improve the detection of genetic variations. Same primer sets were used to amplify the region including V606M and K615N mutations.

Statistical analysis

The Kolmogorov-Smirnov normality test was used to determine the distribution pattern of the variables. All data were presented as mean±standard deviation for continuous variables as appropriate, and number (percentage) for categoric variables. Associations between categoric variables were determined using chi-square tests. Differences between two groups were compared with unpaired Student's t-test for continuous variables. Statistical significance for all tests was accepted at the $p<0.05$ level. Statistical calculations were performed with GraphPad InStat version 3.10, GraphPad Software, San Diego California, USA.

Results

Clinical evaluations of patients

Sixty-nine patient with HCM (32 woman, 37 men, mean age: 50±13.6 years) were included in the study. Most commonly (59% of patients) hypertrophy involved the ventricular septum but not the LV free wall. Twenty-two patients were diagnosed to have concentric type of HCM. In rest of the patients hypertrophy was confined to the midventricular region (2 patients) and anterior portion of the ventricular septum (1 patient). Two patients had both the septal and apical hypertrophy. Interestingly apical and midventricular hypertrophy were seen in only female patients. Mean interventricular septal thickness was 2.20±0.39 cm in the HCM group and there was no significant difference in septal thickness between female and male patients ($p>0.05$). Mean LV posterior wall thickness was 1.31±0.29 cm, found to be significantly thicker in male patients ($p<0.05$) (Table 3). Twenty-seven patients were diagnosed with obstructive variant of HCM. Family history of HCM with sudden cardiac death was present in 6 patients (Table 4).

Frequencies and phenotypic expressions of the mutations

None of the patients in the HCM group were carrying the index mutations. Interestingly, a 40 years old, male, healthy subject from the control group was found to be heterozygous for the

Table 3. Interventricular septum (IVS) and left ventricular posterior wall thickness in study and control group. Data represented as mean echocardiographic value±standard deviation

	Study group			Control group			*P*	
	Women* (n=32)	**Men* (n=37)**	**Total* (n= 69)**	**Women (n=25)**	**Men (n=25)**	**Total* (n= 50)**	***P****	***P******
IVS thickness	2.21±0.44 cm	2.20±0.35 cm	2.20±0.39 cm	0.79±0.5 cm	0.81±2.4 cm	0.83±0.1 cm	<0.0001	0.9485
LVPW thickness	1.39±0.25 cm	1.4±0.29 cm	1.31±0.29 cm	0.78±0.05 cm	0,81±0.09 cm	0.8±0.07 cm	<0.0001	0.0256
p* indicates statistical significance of the difference between study and control group. p** indicates statistical significance of the difference between female and male patients								

Table 4. Clinical characteristics of patients with HCM. Frequency (%) of symptoms, family history and location of hypertrophy in patients were represented in the table

	Patients with HCM		
Characteristics	**Women (n=32)**	**Men (n=37)**	**Total (n= 69)**
Frequency (%)			
Syncope	9.37%	10.25%	9.85%
Obstruction	48%	30%	38.02%
Family history of sudden cardiac death	28.12%	21.6%	24.63%
Family history of syncope	3.12%	8.10%	5.79%
Family history of HCM	31.25%	24.32%	27.53%
Without family history of HCM symptoms	56.25%	51.35%	55.07%
Location of hypertrophy			
Septum	68.7%	47.3%	59%
Concentric	15.6%	39.4%	32.8%
Apical	3.1%	0%	1.49%
Midventricular	3.1%	0%	1.49%
Septum+apical	3.1%	2.6%	2. 9%

R663H mutation (Fig. 3.). His IVS thickness (1.1 cm) and left ventricular posterior wall (LVPW) thickness (1.1 cm) were mildly abnormal according to the normal reference ranges [IVS thickness and LVPW thickness: 0.6-1.0 cm: normal, 1.1-1.3 cm: mild left ventricular hypertrophy (24)]. Allele frequencies for R663H (G/A) mutation were p=0.99/q=0.01 in control group and p=1/q=0 in study group. The prevalence of the R663H mutation was found to be 2% in control group. The allele frequencies for R403Q (G/A), R403L (G/T), R403W (C/T), V606M (G/A) and K615N (G/C) were p=1/q=0 in control and study group. There was no significant difference in the prevalence of the mutations between the HCM and control groups (p>0.05).

Discussion

The aim of this study was to detect the frequency of R403QLW, V606M, K615N, and R663HCS mutations in beta-myosin heavy-chain gene (MYH7) and determine the genotype- phenotype correlations in Turkish patients with HCM. Although we did not find any mutation in patient group, one subject in control group was heterozygous for the R663 mutation. Further, echocardiographic values of this individual were found to be mildly abnormal. The genetic basis for HCM in Turkey has not been extensively studied in large cohort of patients yet and the prevalence of HCM in Turkish population is still unknown. DNA-analysis for mutant genes is the most definitive method for establishing the diagnosis of HCM because of the disease's phenotypic heterogeneity (2, 25). We performed a mutation analysis of six HCM-associated mutations [R403Q (G>A), R403L (G>T), R403W (C>T), V606M (G>A), K615N (G>C), R663H (G>A)] using Real Time PCR and HRM technology in 69 Turkish HCM patients. Especially because of the genetic heterogeneity of HCM, frequencies of these mutations are still unknown in any population. Best of our knowladge, there are only two distinct studies investigating the MYH7 mutations in patients with HCM in Turkey. One of these studies examined the role of R403Q mutation in sudden cardiac death in Turkish patients with HCM and their relatives (32 patient with HCM, n=128) and found that 25 percent of patients were carrying the mentioned mutation, however the presence of the mutation was not significantly associated with sudden cardiac death (26). In the other study, four mutations (R403Q, R453C, R719W, R719Q) were investigated in MYH7 gene and similar to our results none of the mutations were found in patients (n=18) with HCM (27). The R403QLW mutations occurs in close proximity to actin-miyosin interface of the myosin motor domain (28) greatly decrease the actin translocating activity (19) and were associated with high incidence of morbidity and early mortality (29-31). Although 26 percent of our study population have a family history of sudden cardiac death, we could not find the R403Q mutation in any of the patients. These subjects are probably carrying another HCM-causing malign mutation. Val606 is located at the Subfragment 1 which contain a part of actin binding interface of myosin molecule (32, 33) and mildly affects myosin motor and enzymatic activities (19). The V606M mutation is typically associated with a benign form of HCM (20) although an unfavorable prognosis has been reported in several families with the V606M (34, 35). The K615N mutation was suggested to be a cause of HCM as this mutation has been found at the residue conserved through birds to humans (21). Mutations in MYH7 can also cause electrophysiological abnormalities as in the connection between R663H mutation and high risk of atrial fibrillation (AF) in patients with HCM (36). R663H mutation also can cause increased LV mass in hypertrophied individuals (37). In our study, none of the patients in the HCM group were carrying the index mutations. This result could be explained with genetic

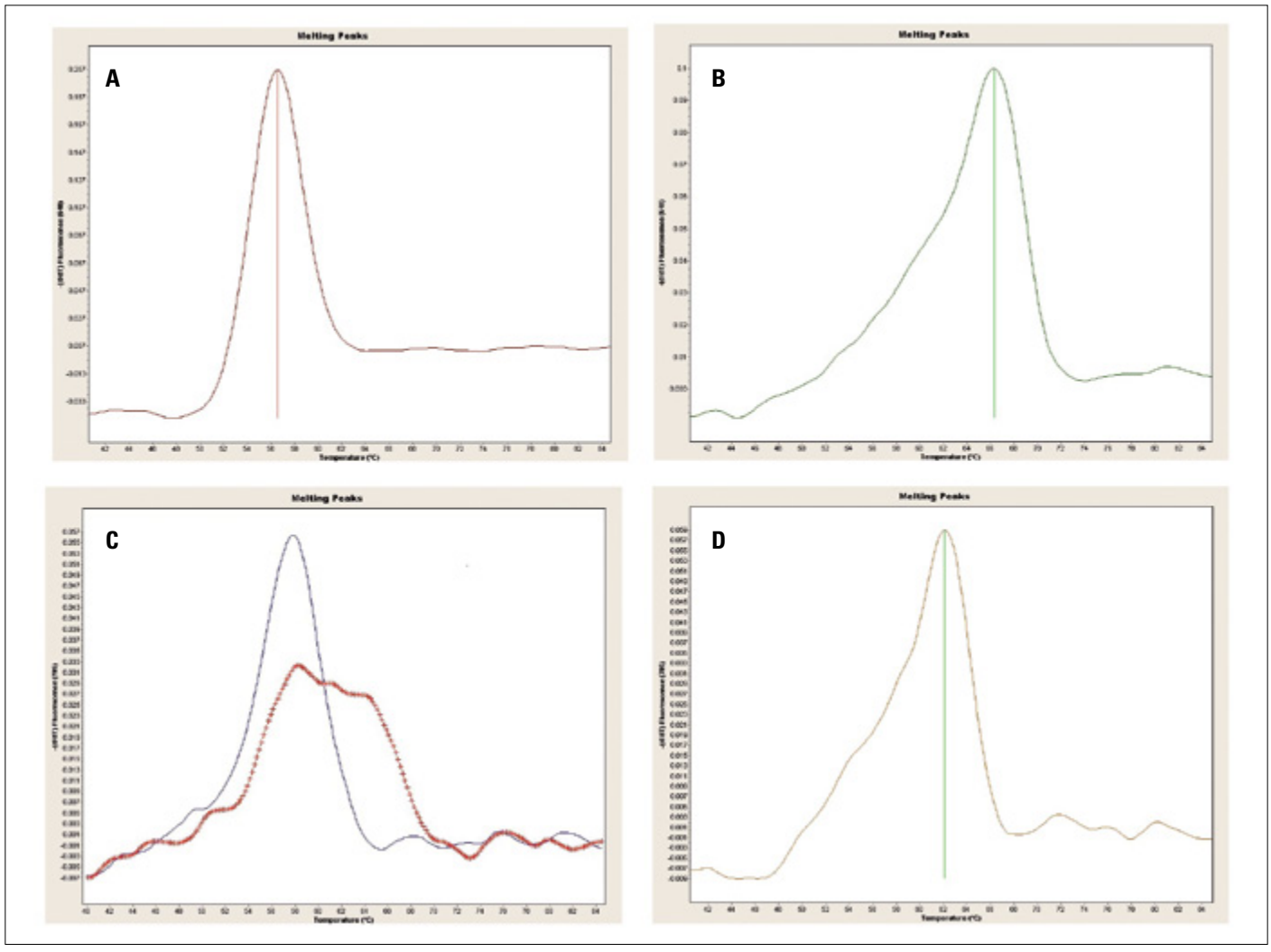

Figure 3. Melting curves of missense mutations. (A) R403QLW,Wild Type (CGG), (B) V606M, Wild Type (GTG), (C) R663H Wild Type (CGC), Heterozygous (CGC/CAC) (D) K615N Wild Type (AAG)

heterogeneity of HCM and incomplete, time-dependent, variable expression of the HCM phenotype. The mutations could be very rare, could be a cause of SCD at very young age or could be a cause of late-onset HCM in Turkish patients. It should be noted that the presence of an HCM-causing mutant gene may not cause LV hypertrophy all the time (2). Therefore, considering the patients with asymptomatic type of HCM couldn't be included in this study, mutations which investigated in this study could be the cause of asymptomatic type of HCM in Turkish patients. Interestingly, one healthy subject from the control group was carrying the R663H mutation with mildly abnormal IVS and LVPW thickness (1.1 cm). This subject could be with asymptomatic type of HCM or there may be no association between HCM and R663H mutation in Turkish patients. Sudden death occurs more commonly in young asymptomatic or only mildly symptomatic patients (38-41), thus the patient was counseled about the risk of HCM and the importance of having regular health examinations.

To our knowledge, this is the first study that has investigated HCM-causing MYH7 mutations by real time PCR and HRM in Turkey. Real time PCR and HRM was proven to be a technique with high sensitivity and low false positive ratio allowing a rapid, innovative and low cost genotyping of HCM. But considering genetic heterogeneity of HCM, by determining the sequence of individual HCM-associated gene, larger genetic regions, full chromosomes or entire genome, more HCM-causing mutations can be identified.

Study limitations

The relatively small study population size is the primary limitation. It should be noted that the study does not include asymptomatic type of patients with HCM. The small number of mutations investigated is also another study limitation. Thus, these findings cannot be generalized to the broader community based on this study alone.

Conclusion

We performed a mutational screening of 6 HCM-associated mutations in 69 Turkish HCM patients and 50 healthy control

subjects. One healthy subject from the control group was found to be heterozygous for the R663H mutation with mildly abnormal IVS and LVPW thickness. There was no significant difference in the prevalence of the mutations between the patients with HCM and the healthy controls (p>0.05).

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