The prevalence of genetic variations in patients with hypertrophic and dilated cardiomyopathy

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INTRODUCTION

Cardiomyopathies are generally defined as myocardial disorders in which the heart muscle is structurally and functionally abnormal, in the absence of coronary artery disease, hypertension, valvular disease and congenital heart disease sufficient to cause the observed myocardial abnormality.[1] According to the morphological and functional phenotype the diagnosis of hypertrophic and dilated cardiomyopathy can be established. Hypertrophic cardiomyopathy (HCM) is an autosomal dominant cardiac disorder[2] with a prevalence of 0.2% in the general population. More than 70% of HCM cases are familial. Hypertrophic cardiomyopathy represents one of the the most frequent causes of sudden cardiac death in the young, especially in competitive athletes[3] and a major cause of morbidity and mortality in the elderly.[4]

Dilated cardiomyopathy (DCM) is an inherited or acquired disease characterized by left ventricular dilatation and reduced systolic function. DCM represents the third most common cause of heart failure and the most frequent cause of heart transplantation. It accounts for approximately 3% of all sudden cardiac deaths in young athletes.[5] Importantly, 30-50 % of all cases are diagnosed as a familial form of DCM.

Recently, more than 630 mutations in 16 different genes have been reported to cause cardiomyopathies. Of these mutations, HCM has been associated with 550 and DCM with more then 52 mutations.[6] In the vast majority of cases these genes encode for sarcomeric contractile proteins: β-myosin heavy chain *(MYH7)*, myosin binding protein C *(MYBPC3)*, troponin T *(TNNT2)*, troponin I *(TNNI3)*, cardiac α-actin *(ACTC)* and α-tropomyosin *(TPM1)*.[3,7] Nearly all of the mutations (86%) are single nucleotide mutations, which can lead to the changes in protein chains. Remaining mutations include small in-frame insertions or deletions and rarely large deletions.[6]

AIM OF THE STUDY

The mutations in both *MYH7* and *TNNT2* genes represent the majority of currently identifiable disease-causing mutations of hypertrophic and dilated cardiomyopathy. The aim of the study was to analyze both *MYH7* and *TNNT2* exons in the patients with HCM and DCM diagnosis to improve the diagnostic and genetic consultancy in affected families.

METHODS

Patients
174 unrelated Caucasian patients with HCM (n=84) and DCM (n=90), mean age 48.4 ± 15.1 years, were evaluated in the Clinical Department of Cardiology and Angiology, First Faculty of Medicine and General University Hospital, Charles University, Prague, Czech Republic, and were included in this single center study.

Samples
Blood samples were collected via puncture of the cubital vein. Blood samples were stored at 4°C and isolation of DNA was performed by a modified salting out procedure according to Miller et al.[8]

***TNNT2* and *MYH7* screening**
First, screening for mutations in *TNNT2* gene exons 7 (I76N) and 8 (R92W) and *MYH7* exons 13 (R403L) and 18 (L663S) was performed using restriction fragment length polymorphism (RFLP) analysis.
Results were confirmed by DNA sequencing.

***TNNT2* sequencing**
The entire coding sequences of *TNNT2* gene were amplified by PCR. Both strands of purified DNA fragments were then sequenced in CEQ 8000 genetic analysis system (Beckman Coulter, CA, USA) according to the manufacturer's protocol.

RESULTS

The mutations I79N, R92W, R92G, R92L in the *TNNT2* gene and mutations R403L, R403Q, R403W, R663S, R663C in the *MYH7* gene were screened by RFLP analysis and results were then confirmed by DNA sequencing. Within our study group consisted of 174 patients (84 patients with HCM and 90 patients with DCM), we identified one R92W mutation in exon 8 of the *TNNT2* gene in a patient with HCM.
We additionally examined all of the 15 exons and their flanking regions of the *TNNT2* gene in the same group of patients. Using DNA sequence analysis to investigate polymorphisms, small deletions and new mutations, we found genetic variations in exon regions in 56 patients and genetic variations in intron regions in 164 patients **(Table 1)**. We confirmed the presence of a unique mutation R92W (exon 8) in a single HCM patient **(Figure 1 and 2)** and another unique mutation A172S (exon 10) was found in a single DCM patient **(Figure 2)**.
The frequencies of remaining *TNNT2* gene polymorphisms from **Table 1** correlated with data in the SNP database (dbSNP) of the National Centre for Biotechnology Information **(Table 2)**. No mutations or polymorphisms were identified in the *MYH7* gene in HMC or DCM patients.

Figure 1. Genetic screening of the *TNNT2* gene (exon 8) by RFLP analysis in the group of patients with HCM.

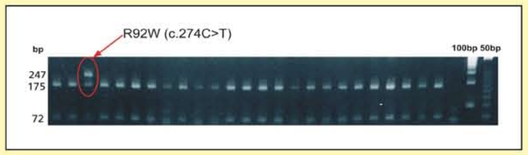

Figure 2. R92W mutation of the *TNNT2* gene was confirmed by DNA sequencing analysis **(A).** Another mutation A172S was found in exon 10 of the *TNNT2* gene in a single patient with DCM **(B)**.

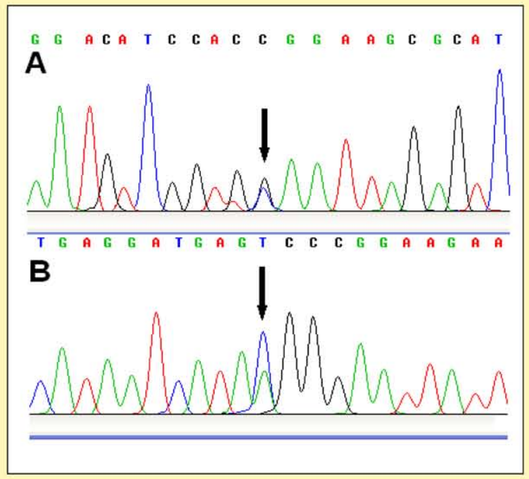

Table 1. Genetic variations (mutations, polymorphisms, small deletions) of *TNNT2* and *MYH7* gene in HCM and DCM patients.

Gene	Exon	Amino acid position	dbSNP access number	DNA variation	Index of patient
TNNT2	7	S79S	rs3729845	aTCG→TCA	9
		I79N	CM951217	ATC→AAC	0
	8	R92W	CM971501	cCGG→TGG	1
		R92G	CM951218	CGG→CAG	0
		R92L	CM961373	CGG→CTG	0
		I116I	rs3729547	ATC→ATT	41
	10	E160	CD951865	deletion GAG	0
		A172S	CM043107	gGCC→TCC	1
	13	K260R	rs3730238	gAAG→AGG	4
	Intron				
	1	-	rs868407	C→T 74	
	2	-	rs45533739	deletion CTTCT	70
	12	-	rs2275861	C→T 20	
MYH7	*Exon*				
	13	R403L	CM930503	CGG→CTG	0
		R403Q	CM900168	CGG→CAG	0
		R403W	CM930504	tCGG→TGG	0
	18	R663S	CM0312273	gCGC→AGC	0
		R663H	CM993620	CGC→CAC	0
		R663C	CM973126	gCGC→TGC	0

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Table 2. *TNNT2* gene polymorphisms and small deletions in HCM patients.

		Genotypes (%)			*Allele frequencies*	
Exon 7	***S79S***	***GG***	***GA***	***AA***	***G***	***A***
		89	11	0	0.946	0.054
Exon 8	***I116I***	***TT***	***CT***	***CC***	***T***	***C***
		45	40	15	0.653	0.347
Exon 13	***K260R***	***AA***	***AG***	***GG***	***A***	***G***
		95	5	0	0.975	0.025
Intron 1	***C/T***	***TT***	***CT***	***CC***	***T***	***C***
		49	39	12	0.685	0.315
Intron 2	***deletion CTTCT***	***++***	***+-***	***--***	***+***	***-***
		34	49	17	0.589	0.411
Intron 12	***C/T***	***TT***	***CT***	***CC***	***T***	***C***
		77	23	0	0.883	0.117

CONCLUSIONS

The limited genetic screening analysis is not suitable for routine testing of disease-causing mutations in patients with HCM and DCM as only individual mutation-positive cases may be identified. Therefore, this approach cannot be recommended for daily clinical practice even though it currently represents the only available strategy in majority of cardio-centers due to financial reasons. More cost-effective methods enabling wide genome screening are promising and should be implemented in genetic analyses of cardiomyopathies in near future.

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