RESEARCH NOTE

Novel mutations in beta-myosin heavy chain, actin and troponin-I genes associated with dilated cardiomyopathy in Indian population

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Introduction

Dilated cardiomyopathy (DCM) represents a large subset of congestive heart failure cases with a risk of sudden cardiac death (Kamisago *et al*. 2000). It is a primary myocardial disease and genetically heterogeneous disorder, characterized by progressive systolic dysfunction due to cardiac chamber dilatation and inefficient myocardial contractility. The phenotype in idiopathic/primary DCM (IDCM) is characterized by cardiac muscle dysfunction in the absence of secondary causes and conduction defects such as atrio–ventricular blocks / valvular dysfunction / systemic / skeletal muscular disorders in ischemic/secondary DCM.

Majority of the primary DCM cases are sporadic but a familial transmission (FDCM) is observed in 25%–35% cases, inherited as autosomal dominant/recessive or X-linked trait with variable expressivity and penetrance. Studies have led to the identification of null and/or missense mutations in more than 15 different genes coding for sarcomeric, cytoskeletal, or regulatory proteins as a primary cause of DCM (Olbrich 2001; Murphy *et al*. 2004).

Screening of *MYH7*, cardiac *ACTC* and *TNNI3* genes in 100 primary DCM patients using single stranded conformation polymorphism technique (SSCP) revealed two novel missense mutations (Gly377Arg and Arg787His), seven silent mutations, two polymorphisms in *MYH7* gene, and two novel missense mutations (Arg74Pro and Ala91Thr) and one silent mutation pertaining to *TNNI3* gene. The study revealed majority of the mutations in exons which fall into the protein domain regions that are critical for myocardial contraction initiation and regulation processes.

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Materials and methods

Subjects

Blood samples were collected from 100 IDCM patients referred to various cardiology units of Care hospital, Krishna Institute of Medical Sciences and Niloufer hospital for Children, Hyderabad, India, over a period of three years, after obtaining written consent from each patient. All patients were of south Indian origin. Clinical evaluation of DCM was based on physical examination, chest radiography, electrocardiography and echocardiography. Patients above 25 years of age had undergone cardiac catheterization and coronary angiogram to exclude the possibility of significant coronary artery disease or valvular disease. Whole blood samples from 100 age-matched and sex-matched unaffected healthy individuals with no history/family history of cardio-vascular disorders served as controls.

Detection of mutations

DNA was isolated from whole blood as per the standard protocol (Lahari and Nurunberger 1991). The screening of *MYH7*, *ACTC* and *TNNI3* genes were carried out by PCR based SSCP analysis. Primer sequences of each of the exons were taken from http://genetics.med.harvard.edu/~seidman/lamin.html.

A standard PCR protocol for amplification was followed wherein a 25-μL PCR reaction mixture contained 0.5-μg genomic DNA, 0.5 mM of each dNTP, 1.5–3 mM $MgCl_2$, 50–200 pmol of each of the forward and reverse primer, 10 mM Tris-HCl buffer (pH 8.3), and 1 U *Taq* DNA polymerase at specified annealing temperature for 25 cycles. After a 1 min denaturation step at 94°C, 25 cycles of amplification (denaturation at 94°C for 30 s, primer annealing at 55–65°C for 30 s, and extension at 72°C for 2 min) were carried out.

Keywords. beta-myosin heavy chain; alpha-cardiac actin; cardiac troponin-I; novel sarcomere mutations; codon bias; dilated cardiomyopathy; human genetics.

SSCP analysis was carried out following the protocol of Orita *et al*. (1989) with slight modification. The amplified samples were denatured for 10 min at 95°C and quenched on ice for 5 min prior to loading and then electrophoresed for 8–12 h (depending on the amplimer length) on a 10% non-denaturing PAGE gel at 20 mA at 24°C. The gels were silver stained. The PCR products of all the samples showing band pattern variation were purified using the commercial purification kit (catalog no. 28104; Qiagen, California, USA) and the product was sequenced (sense strand) on an ABI prism 377 DNA sequencer (Macrogen, Seoul, Korea).

Results

In the present study no mutations/SNPs were identified in *ACTC* gene. Two novel mutations were identified in each *TNNI3* and *MYH7* genes.

Mutations

Cardiac beta-myosin heavy chain gene (β-MYH7): The screening of *MYH7* gene led to the identification of two novel missense mutations. A single-nucleotide variant, G>C transversion (GGC → CGC) at nucleotide 9667, (figure 1a) in exon 12, which would substitute an arginine for the normal glycine at residue 377 (Gly377Arg), was found in one DCM patient. The female proband was diagnosed at the age of 11 years, was clinically symptomatic with 30% EF, myocarditis and a history of consanguinity in the family.

A G>A transition (CGT→CAT) was identified at nucleotide 14088 (figure 1b) in exon 21, which replaces arginine with histidine at residue 787 (Arg787His), in another proband. The female patient was diagnosed with DCM at seven years of age. Clinical symptoms include 40% EF, myocarditis, trivial mitral regurgitation with parental consanguinity and a family history of sudden cardiac deaths.

Cardiac troponin-I (TNNI3): The screening of *TNNI3* gene identified two novel mutations. In exon 5 a G>A heterozygous transition (GCG→ACG) was identified at nucleotide 2627, which will result in replacement of alanine to threonine at amino acid 91 (Ala91Thr) (figure 2a). Also, G>C and C>G homozygous transversions were observed at 27 and 28 bases upstream of the exon 5. The female patient was six years old and reported with 30% EF, mild mitral regurgitation and with a history of sudden cardiac deaths in the family.

Another male patient of age 13 years showed a G>C homozygous transversion (CGC→CCC), at nucleotide 2577, which resulted in an Arg74Pro missense mutation (figure 2b) and an intronic G>C transversion at position 27 as well as a C>G transversion at position 28 upstream of the exon 5. The proband had a history of sudden cardiac deaths in the family and clinically showed 25% EF, myocarditis, moderate tricuspid regurgitation with restricted left ventricular filling pattern. The proband died during the course of the study.

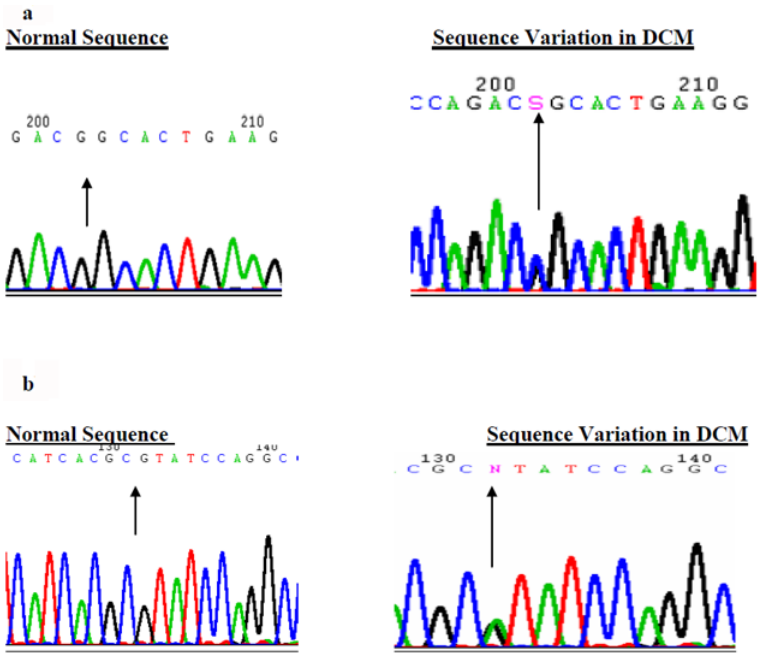

Figure 1. (a) Electrophoregrams corresponding Gly377Arg (GGC → CGC) variation in exon 12 of *MYH7* gene. NCBI accession no. EF630366; b, electrophoregrams corresponding Arg787His (CGT → CAT) variation in exon 12 of *MYH7* gene NCBI accession no. EU091311.

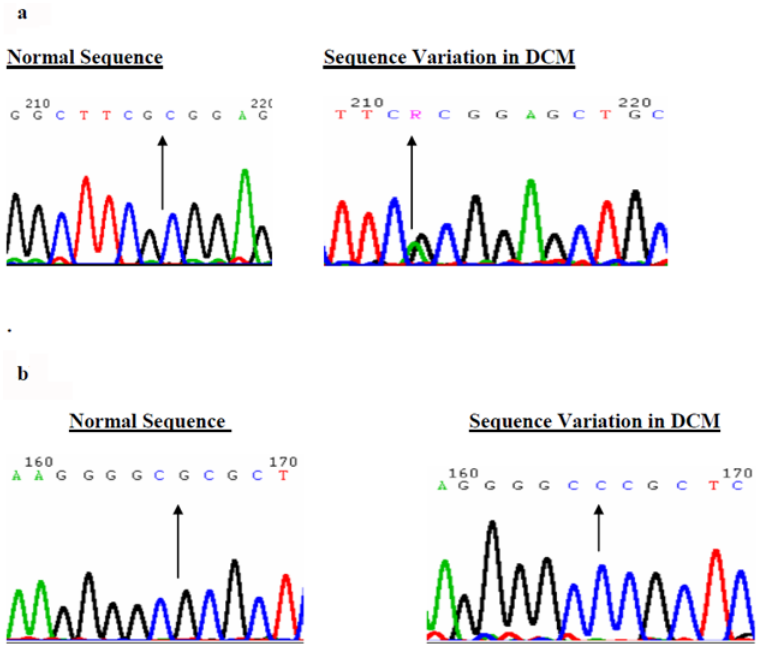

Figure 2. (a) Electrophoregrams corresponding Ala91Thr variation in exon 5 of *TNN13* gene NCBI accession no. EU091318; b, electrophoregrams corresponding Arg74Pro variation in exon 5 of *TNN13* gene NCBI accession no. EU091319.

Polymorphisms

We identified seven novel synonymous base changes/silent mutations in MYH7 gene, in 15 DCM patients. The type of mutation/base change and clinical history of the corresponding patient is given in table 1. In addition, two novel SNPs were identified in six DCM patients and nine control samples. One synonymous base change and two intronic variations were identified in *TNNI3* gene. These intronic variations were also present in both the probands harbouring *TNNI3* mutations (table 1).

Discussion

DCM is characterized by ventricular chamber dilation and wall thinning, which is accompanied by severe systolic contractile dysfunction. Mutations in both sarcomeric as well as cytoskeletal genes have been implicated in the disease. The variable expression and penetrance of each of the genes harbouring different mutations result in vast clinical heterogeneity among the patients.

The globular head domain of myosin is a molecular motor, which utilizes ATP hydrolysis to generate directed movement along actin filaments. It comprises of four major sub-domains within the motor domain: the N-terminal/ATP binding region (residues 4–265), upper 50 kDa (residue 266–380), lower 50 kDa sub-domain/actin binding region (residue 381–729), and the converter/hinge-lever arm region (residue 730–790). A cyclic interaction between myosin and actin provides the driving force for myocardial contraction. The hinge/converter region functions as a hinge that transmits movement and directionality from the head of myosin to the neck, thereby propelling the thick filament (Houdusse *et al*. 1999). Mutations in any of these regions are known to disrupt the myocardial contraction mechanism, which is commonly seen in DCMs.

The phenotype in *MYH7* mutation carriers was similar. Mean age at diagnosis was very early (<12 years), poor EF (<50%), myocarditis. Early age at onset with *MYH7* gene mutations has also been described in earlier reports (Kamisago *et al*. 2000; Daehmlow *et al*. 2002). In a study from north India, a mutation at the 377 amino acid (Gly377Ser) has been reported in DCM, having a familial transmission (Rai *et al*. 2009). Interestingly we found another novel mutation at the same position in south Indian population (Gly377Arg), indicating that any change in this glycine residue is likely to diminish the production of contractile force, tilting the phenotype toward DCM.

The R787H mutation has been previously reported in hypertrophic candiomyopathy (Pascale *et al*. 2003). This is the first case where the same *MYH7* mutation is being reported in DCM. . This same mutation has also been reported in north Indian population in patient with apical hypertrophy (Rai *et al*. 2009). HCM and DCM are considered partially allelic disorders whereby identical genes have been implicated in the pathogenesis of both diseases. Vasile *et al*. (2006) have also reported a DCM associated mutation R975W in metavinculin gene as a cause of HCM, and suggested the important role of modifier genes/environmental stressors in cardiac remodelling that would be critical in

Table 1. Silent mutations identified in dilated cardiomyopathy patients of Indian origin.

Gene	Exon	Mutation per SNPs	Patient clinical symptoms	NCBI accession no.*
β-*MYH7*	7	Ala199Ala	P05: 18% EF, mild MR, altered left ventricular compliance	EF630363
β-*MYH7*	12	Lys365Lys	P06: F/H/O-consanguinity & neonatal deaths. 50% EF, severe MR & TR	EF630364
β-*MYH7*	12	Lys365Lys & Gly354Gly	P07: F/H/O-consanguinity & SCD, 44% EF, myocarditis, severe MR & severe PAH P08: 15% EF, myocarditis, severe MR, dilatation of all cardiac chambers, tachycardia & severe PAH	EF630365
β-*MYH7*	12	Lys365Lys & Ser343Ser	P09: F/H/O-DCM in one sib, and two sibs affected with other cardiacdisorders, F/H/O- SCD, 40% EF, severe MR	EF630367
β-*MYH7*	19	Gly682Gly intron region: A>G homozygous transition at 82 bps downstream of exon 19.	P10 and P11: left auricular dilatation, moderate MR & TR	EU091312
β-*MYH7*	19	Gly682Gly intron region: A>G homozygous transition at 82 bps & 90 bps downstream of exon 19.	P12, 13, 14, 15 and 16: Only the juvenile patients had dilatation in all the four chambers of the heart with moderate LV dysfunction while the two affected adult individuals showed only severe LV dilatation & dysfunction. All the 5 affected showed severe MR & TR	EU091313
TNNI-3	5	Arg68Arg intron region: CG/GC at 27 & 28 bps upstream of exon	P17, 18 and 19: F/H/O- neonatal deaths & SCD. 30% to 33% EF, myocarditis, severe systolicdysfunction, moderate MR & PAH	EU091317
β-*MYH7*	19	A>G homozygous transition at 56 bps and 64 bps upstream of exon 20 i.e in intron 19 region. (typeI19A)	P20, 21, 23 and 24: low EF, mild MR & TR with restricted left ventricle filling pattern. Controls: asymptomatic for the above clinical features	EU091314
β-*MYH7*	19	Del of A at 64 bps upstream of exon 20 & A>G homozygous transition at 86 bps upstream of exon 20 (type I19B)	P22: 15% EF, myocarditis, severe MR, dilatation of all cardiac chambers, tachycardia, and severe PAH Controls: asymptomatic for the abovephenotype	EU091315

*NCB1 GeneBank: www.ncbi.nlm.nih.gov/nuccore.

determining the cardiomyopathic phenotype. Mutations in the same gene and even the same mutation in different background can result in disparate phenotypes (Capell and Collins 2006). This kind of phenotypic plasticity is well documented in case of cardiomyopathy.

The three subunits of the troponin complex are held together by a core of structural, Ca^{2+}-independent interactions between the COOH-terminal domain of TnC, the amino-terminal domain of TnI (TnI_{1-98}), and the last 50 residues of TnT. The amino-terminal domain of TnI interacts with TnT and is involved in the Ca^{2+}-independent interaction with TnC. TnI_{1-98} residues interact with 216–263 of TnT that is essential for the binding of TnI_{1-98}–TnC to the thin filament; and the residues 1–102 of TnI are also required for the binding of TnC/TnI to actin-Tm-TnT in the presence of calcium (Malnic *et al*. 1998). The R74P and A91T mutations lie in the NH2 terminal domain of *TNNI* and are likely to affect the stability of the troponin complex leading to altered regulation of myocardial contractility.

Both the *TNNI3* mutations were associated with early age at onset. In addition both the probands also harboured identical intronic variations 27 and 28 bases (CG/GC) in intron 4 highlighting the significance of the intronic variations in the disease expression.

The present study also reports eight synonymous changes/silent mutations in *MYH7* and *TNNI3* (table 1). It

is well known that a change in primary protein structure can considerably affect the protein folding, function and its final expression. But the role of silent mutations, that do not affect amino acid sequence, in protein folding and function is debatable. Recent theoretical studies have suggested that codon usage is not random and experimental studies in prokaryotes suggest that this may be true (Supek and Vlahovicek 2005). Studies relating to the above have revealed that when there is a codon change in a conserved protein sequence, the timing of cotranslational folding and translational efficacy is affected and this may result in altered function (Anthony and Skach 2002; Josep 2005; Wang and Wolfgang 2006). Also it has been shown that the codon usage frequencies in most of known familial dilated cardiomyopathy gene sequences are consistently biased, and significantly different from the average codon usage frequencies of human genes (Yanhong *et al*. 2005). This finding may be clinically important in terms of the vast phenotypic variation among patients.

Our study revealed 5% of mutations/silent mutations in *TNNI3* gene which is a high frequency considering that only one mutation in this gene is reported in DCM. (Murphy *et al*. 2004) All these variations were found in exon 5, and hence we suggest that this exon could be a mutational hot spot in our population. Also, we report 9% of *MYH7* mutations/silent mutations in Andhra Pradesh population which is significantly higher than 1.6% reported in DCM from northern Indian. This indicates extent of genetic variation among different ethnic groups and the possibility of the abovementioned exons to be mutational hot spots in the population studied implicating the specific domains coded by these exons in the disease pathogenesis. In addition, the considerable variation in the age at onset of the disease, and degree of disease expression, the vast clinical symptoms of individuals affected by the same and/or different mutations clearly suggests the involvement of both genetic as well as non-genetic modifiers such as environment/protein markers in the disease pathogenesis.

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