Genotype phenotype correlations of cardiac beta-myosin heavy chain mutations in Indian patients with hypertrophic and dilated cardiomyopathy

Taranjit Singh Rai · Shamim Ahmad · Ajay Bahl ·
Monica Ahuja · Tarunveer Singh Ahluwalia ·
Balvinder Singh · K. K. Talwar · Madhu Khullar

Abstract The aim of the current study was to determine the frequency of mutations in the beta-myosin heavy chain gene (MYH7) in a cohort of hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) and their families, and to investigate correlations between genotype and phenotype. About 130 consecutive patients diagnosed with HCM or DCM (69 with HCM and 61 with DCM) attending the cardiology clinic of Post Graduate Institute of Medical Education and Research were screened for mutations in the *MYH7* gene. The control group for genetic studies consisted of 100 healthy subjects. We report 14 mutations in 6 probands (5 probands in HCM and 1 proband in DCM) and their family members. Out of these 6 mutations, 3 are new and are being reported for the first time. One known mutation (p.Gly716Arg) was found to be "de novo" which resulted in severe asymmetric septal hypertrophy (31 mm) and resulted in the sudden cardiac death (SCD) of the proband at the age of 21 years. Further, a DCM causing novel mutation p.Gly377Ser was identified which resulted in the milder phenotype. The present study shows that there is genetic and phenotypic heterogeneity of cardiomyopathies in Indian population. Further, the location and type of mutation in a given sarcomeric gene determines the severity and phenotypic plasticity in cardiomyopathies.

Keywords Beta myosin heavy chain · Hypertrophic cardiomyopathy · Dilated cardiomyopathy · Mutation

T. S. Rai · M. Ahuja · T. S. Ahluwalia · M. Khullar (✉)
Department of Experimental Medicine and Biotechnology, Post Graduate Institute of Medical Education and Research, Chandigarh, India
e-mail: madhu.khullar@gmail.com

S. Ahmad · A. Bahl · K. K. Talwar
Department of Cardiology, Post Graduate Institute of Medical Education and Research, Chandigarh, India

B. Singh
Institute of Microbial Technology, Chandigarh, India

Introduction

Cardiomyopathies are primary disorders of cardiac muscle associated with abnormalities of cardiac wall thickness, chamber size, contraction, relaxation, conduction, and rhythm. They are a major cause of morbidity and mortality at all ages and often result in heart failure. Over the past two decades, molecular genetic studies of humans and analyses of model organisms have made remarkable progress in defining the pathogenesis of cardiomyopathies. Hypertrophic cardiomyopathy has been found to result largely from mutations in sarcomere genes, whereas dilated cardiomyopathy is mainly caused by mutations in genes encoding contractile, cytoskeletal, and calcium regulatory proteins. Dissecting the complex genetic basis of different phenotypes of cardiomyopathy may be key to both better understanding and optimally managing these cardiovascular diseases. The discovery of causative mutations has important implications for diagnosis, prognosis, and intervention. India, with a population of more than a billion, has a large number of patients affected with cardiomyopathies; however, there is little information on spectrum of gene mutations and their association with disease phenotype and prognosis. In the present study, we have prospectively

examined the spectrum of *MYH7* mutations in 130 consecutive patients of HCM and DCM.

Material and methods

Patients

About 130 consecutive patients diagnosed with HCM and DCM (69 with HCM and 61 with DCM) attending the cardiology clinic of Post Graduate Institute of Medical Education and Research, were enrolled in the study after obtaining informed consent from all the participants. This study was approved by the Ethics committee of the institute. All the patients underwent physical examination, 12-lead ECG, and trans-thoracic two-dimensional echocardiography and Doppler studies.

About 100 healthy controls were also enrolled for the study.

Echocardiography

Standard views for M-mode and two-dimensional studies were obtained. For HCM patients end diastolic left ventricular wall thickness was recorded at the level of the mitral valve and papillary muscle in the anterior, posterior, septal, and lateral walls using short-axis two-dimensional images. Anterior and posterior septal thickness at the apex was assessed from apical short-axis view. The maximum left ventricular wall thickness was defined as the maximal measurement recorded in any of the myocardial segments studied. Left ventricle inflow and outflow velocities were determined using continuous and pulse wave doppler echocardiography.

Hypertrophic cardiomyopathy

Inclusion criteria

Individuals were diagnosed with HCM when echocardiography identified unexplained left ventricle hypertrophy greater than or equal to 13 mm or >2 standard deviations corrected for body surface area for children and adolescents.

Exclusion criteria

Patients with systemic hypertension and aortic stenosis or any other systemic or cardiac disease capable of producing the magnitude of wall thickening were excluded from the disease.

Dilated cardiomyopathy

Inclusion criteria

1. Left ventricular ejection fraction (LVEF) ≤40% on echocardiography
2. Absence of past history of myocardial infarction or coronary artery disease.
3. Absence of secondary cause of left ventricular dysfunction including primary valvular heart disease, ventricular outflow tract obstruction, and coronary artery anomalies.
4. Coronary angiography was carried out in all patients more than 35 years of age and in young individuals if clinically indicated.

Exclusion criteria

Patients with concomitant disease like infection, autoimmune disease, cancer, as well as patients with coronary artery disease (CAD) and with advanced chronic renal failure (CRF) were excluded.

Amplification of genomic DNA

The DNA was prepared from peripheral blood leukocytes by proteinase-phenol -chloroform extraction method. Primers for amplification reactions were designed by Primer3 software. Exons 8–24 of *MYH7* gene (Accession no. M57965.2) were amplified using intronic primers flanking these exons according to the reported sequences of the *MYH7*. Primer sequences and PCR amplification conditions are available on request.

Denaturing high performance liquid chromatography (DHPLC)

After PCR, the amplified samples were directly analyzed by DHPLC (Transgenomic WAVE system Model 4500) using UV detection. Eight microliters of the PCR product was injected to reverse-phase DNASepR cartridges (Transgenomic USA). PCR products were eluted from the column by a linear acetonitrile gradient in 0.1 mM TEAA buffer (Transgenomic, USA), pH 7 at a constant flow rate of 0.9 ml/min using Rapid DNA programme. Run time was 4 min per sample. These conditions were used based on WAVE-MAKER software (Supplementary online materials).

Sequencing

Variants identified by DHPLC were amplified again and PCR products were purified prior to sequencing using a kit

from Amersham Pharmacia Biotech. Sequencing was performed using a Big Dye Terminator Cycle Sequencing Kit from Applied Biosystems Inc. The sequences were analyzed on an ABI Prism 3100 Sequencer in accordance with the manual of the manufacturer. The identified mutation was reconfirmed by taking a second blood sample from the affected patient and reanalyzing the amplified PCR product by sequencing (Supplementary online materials).

Statistical analysis

All statistical analyses were performed using SPSS (Statistical Package for Social Sciences) for windows (Version 10). Discrete and continuous variables were compared between patients and controls using Pearson's χ^2 test and unpaired t-test as appropriate. P values were subject to Bonferroni's correction and considered significant when <0.05.

Results

Clinical characteristics

The clinical characteristics of the patients are given in Table 1. At presentation 25.86% of the patients were in New York Heart Association (NYHA) class III and IV. While 67.24% patients had dyspnea, 56.89% had angina pectoris and 25.28% of the patients had at least one event of syncope. ECG was abnormal in 64% of HCM patients and 72% of DCM patients. The mean septal thickness was 22.68 ± 5.17 mm for HCM patients. The mean LVEF at enrollment was $37.57 \pm 12.09\%$ for DCM patients.

Mutations

We identified 6 MYH7 mutations (5 in HCM and 1 in DCM). Table 2 shows the location, type, and associated phenotype of these mutations. Out of these 6 mutations, 3 are new and are being reported for the first time. One known mutation was found to be "de novo."

Genotype phenotype correlations

p.Tyr266Cys

This mutation was observed in Proband H47. Echocardiography of the proband (Fig. 1) revealed severe left ventricular hypertrophy with septal thickness of 32 mm and involvement of right ventricle. This mutation has been identified for the first time in an HCM proband. The mutation lies in the conserved N-terminal region of MYH7

Table 1 Demographic profile of cardiomyopathy patients

Characteristics (all patients)	$N = 130$
Age (years)	47.48 ± 2.30
Male sex (%)	66
NYHA class III or IV (%)	25.86
Dyspnea (%)	67.24
Angina pectoris (%)	56.89
Syncope (%)	25.28
HCM	$N = 69$
LVEDD (mm)	35.00 ± 6.89
LVESD (mm)	18.29 ± 6.89
Septum (mm)	22.68 ± 5.17
Abnormal ECG (%)	64
DCM	$N = 61$
LVEF at enrollment:	37.57 ± 12.09
Abnormal ECG (%)	72

and results in substitution of tyrosine with cysteine. This mutation lies on the junction of exon 9 and 10, so it might be affecting the splicing of the pre mRNA of MYH7 which results in expression of aberrant MYH7 protein. The contractility of heart depends upon various sarcomeric proteins which work in a harmonious way. A mutant MYH7 leads to disruption of this harmony and hence results in a hypertrophied response. This was also evident from the observed phenotype of the proband. She had severe left ventricular hypertrophy with the involvement of right ventricle, indicative of a severe phenotype. Bioinformatic analysis showed that this mutation lies in the upper 50 kDa domain which is involved in the actin binding. The proper interaction of actin and myosin is very important for the ATPase activity of the myosin and for pulling of actin thin filaments by myosin. Any disruption of this interaction results in abnormal pulling of thin filaments over thick filaments and hence abnormal contractility. This leads to hypertrophic response as was seen in the case of present proband with this mutation.

We could not confirm if this mutation was familial or "de novo" as other family members of the proband were not available for genotyping.

p.Gly377Ser

This mutation was seen in Proband D89. Echocardiography of the proband revealed dilated ventricles. Family history revealed sudden cardiac death (SCD) of the father of the proband who died suddenly at the age of 50 years. The mutation lies in the conserved N terminal region of MYH7 and results in substitution of glycine with serine. Five other

Table 2 *MYH7* mutations and their associated phenotypes

Exon	Type	Status	Location in *MYH7*	Phenotype	Maximal septal thickness (mm)
10	p.Tyr266Cys	Novel	Upper 50 kd domain	Severe LVH (max 32 mm) with RVH	32
12	p.Gly377Ser	Novel	Upper 50 kd domain	DCM recovered	LVEF at enrollment = 32%
15	p.Met515Thr	Novel	Relay	ASH, SCD	19
16	p.Val606Met	Known	Upper 50 kd domain	Concentric LVH (24 mm)	24
19	p.Gly716Arg	Known	Converter	Severe ASH (max 31 mm)	31
21	p.Arg787His	Known	Neck	Apical hypertrophy	18

ASH Asymmetrical septal hypertrophy; *LVH* Left ventricular hypertrophy; *RVH* Right ventricular hypertrophy; *LVEF* Left ventricular ejection fraction
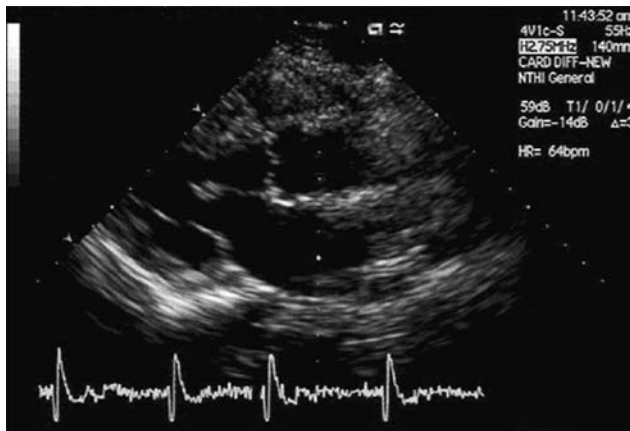

Fig. 1 Echocardiography of the proband H47 showing severe left ventricular hypertrophy

Fig. 2 Pedigree of Proband D 89: only generations III and IV were available for genotyping, Filled symbol with arrow is proband, partially filled symbols are asymptomatic carriers, slashed is deceased individuals, ? = status unknown

family members aged 38, 28, 20, 15, and 12 years were found to be carriers of this novel mutation and were phenotypically normal (Fig. 2). Hence, the mutation was classified as familial. The LVEF of the patient has improved from 32% at presentation to 52% presently. Since several young members were identified as carriers and they are disease free, the mutation appears to be associated with a variable penetrance and favorable disease course. The absence of disease in the mutation carriers shows that other environmental factors and disease modifiers are involved in the pathogenesis of the dilated cardiomyopathy. The absence of disease in the mutation carriers shows that other environmental factors and disease modifiers are involved in the pathogenesis of the dilated cardiomyopathy.

p.Met515Thr

This mutation was identified in proband H10. Echocardiography of the proband with p.Met515Thr mutation revealed that she had asymmetrical septal hypertrophy with septal thickness of 19 mm. Echocardiography video of this proband was not available. She died at the age of 20 due to sudden cardiac death. The mutation lies in the relay domain
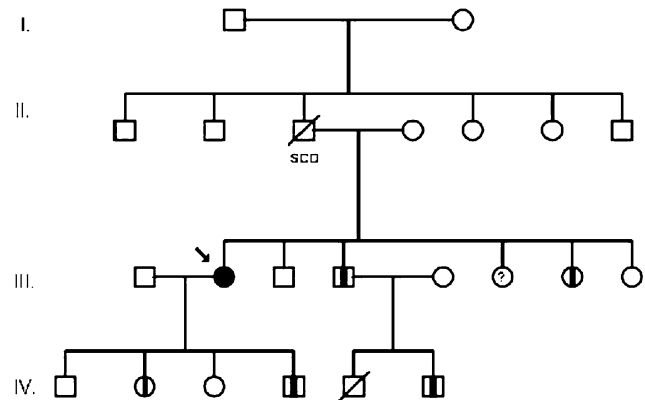
of *MYH7*. During the recovery stroke, the myosin motor is primed for the next power stroke by a 60° rotation of its lever arm [1]. This reversible motion is coupled to the activation of the ATPase function of myosin through conformational changes along the relay helix, which runs from the Switch-2 loop near the ATP to the converter domain carrying the lever arm. The flexibility changes in the converter and relay domains result in the anomalous ATPase function of *MYH7*. The mutation appears to be associated with high risk of sudden cardiac death. A previously described different mutation Met515Arginine has also been reported at the same position by Van Driest et al. [2]. The phenotype was not described in the reference. The family of the proband was not available for the study.

p.Val606Met

This mutation was identified in proband H74. Echocardiography of the proband (Fig. 3) revealed concentric left ventricular hypertrophy with septal thickness of 24 mm. Pedigree of the family is shown in Fig. 4. This is a known mutation. It results in substitution of valine with methionine. It lies in the upper 50 kDa domain. Both the children
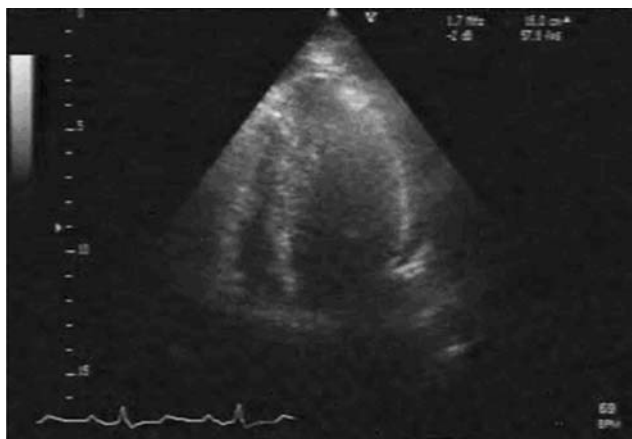
Fig. 3 Echocardiography of the proband showing concentric left ventricular hypertrophy
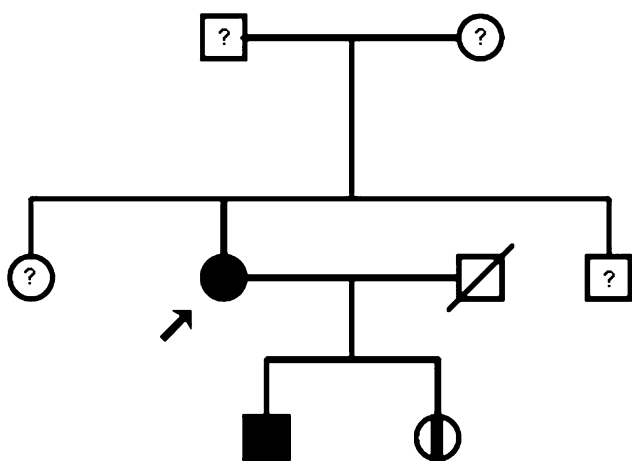

Fig. 4 Filled symbol with arrow is proband, filled symbol is symptomatic carrier, partially filled symbols are asymptomatic carriers, slashed is deceased individuals, ? = status unknown

of the proband were found to be carriers of the mutation. Hence, the mutation was classified as familial. On evaluation, the son of the proband, aged 25 years was found to have breathlessness on exertion and climbing two floors. Echocardiography of the son (Fig. 5) showed that he had HCM with maximal septal thickness of 13 mm. The daughter of the proband aged 19 years was found to be asymptomatic with maximal septal thickness of 9 mm.

There have been conflicting reports regarding phenotypic association of this mutation. A few studies have observed this mutation to be associated with mild phenotype and as the prototype of a "benign" *MYH7* mutation [3]. However, Fannapazir et al. [4] reported this mutation to be associated with severe phenotype: a family with four out of eight affected carrier members died of SCD. Nakajima et al. [5] also reported several premature deaths in persons with this mutation. So there, is large variability of phenotypes associated with this mutation. This may be

Fig. 5 Echocardiography of the proband's son

due to the effect of modifier genes and environmental factors. Based on the previous published literature and our results, the mutation appears to be associated with variable phenotypic expression of the disease.

p.Gly716Arg

This mutation was identified in proband H91. Echocardiography of proband (Fig. 6) revealed severe asymmetrical septal hypertrophy with septal thickness of 31 mm. Pedigree of the family is shown in Fig. 7. Both father and mother of the proband were not found to carry this mutation; hence, the mutation was classified as "de novo." Paternity was confirmed by STR analysis of 15 microsatellite markers. The proband died due to SCD at the age of 21 years.

This is the first time that this mutation has been found to be "de novo." This suggests that mutations in the sarcomeric genes can be familial as well as "de novo" and certain regions in these genes are more prone to mutations and occur as "hot spots." There are consistent reports
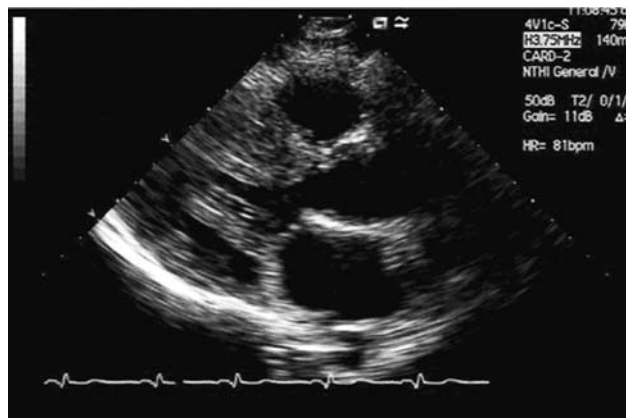

Fig. 6 Echocardiography of the proband showing severe asymmetrical septal hypertrophy
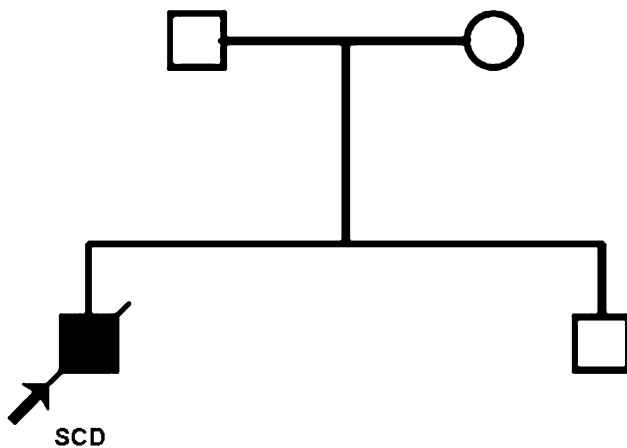
Fig. 7 Pedigree of Family H91 with p.Gly716Arg mutation. Filled symbol with arrow is proband. SCD is sudden cardiac death

Fig. 8 Echocardiography of the proband showing apical hypertrophy

suggesting phenotypic associations of this mutation with severe phenotype. Hwang et al. [6] reported four SCD in 13 affected individuals in a family. Van Driest et al. [2] also reported sudden cardiac death at a young age in a patient with this mutation. Based on the previous published literature and our results, the mutation appears to be associated with severe asymmetrical hypertrophic cardiomyopathy and high risk of SCD. The mutation lies in the converter domain of *MYH7*. The rotation of the converter domain is essential for ATP hydrolysis. The flexibility changes in the converter domain results in the anomalous ATPase function of *MYH7* and is expected to lead to a severe phenotype. The mechanisms through which this mutation leads to severe phenotype remains to be examined in vivo.

p.Arg787His

This mutation was identified in proband H61. The proband was diagnosed during routine examination for abnormal ECG. This is a known mutation [7]. It results in substitution of arginine with histidine. It lies in the neck domain of *MYH7*. The phenotype of the mutation carrier was not described in the previous report [7]. Echocardiography of the proband (Fig. 8) revealed apical hypertrophy with a maximum thickness of 18 mm. The mutation appears to be associated with favorable disease course. The proband did not participate in further screening process. Only sample A of the proband was tested. Because of the non-availability of the family of the proband for genetic analysis, we could not classify this mutation as familial or "de novo."

Discussion

Dissecting the complex genetic basis of cardiomyopathy is important for both better understanding and optimally managing these genetic cardiovascular diseases. Very little data are available on spectrum of mutations, genotype–phenotype correlations, and prognosis in Indian patients with mutations in MYH7 gene. Till date, only two *MYH7* mutations, homozygous R870H and *MYH7* DeltaE927, have been reported from HCM patients from South India [8, 9].

We observed allelic heterogeneity of MYH7 mutations; 2 MYH7 mutations at different locations resulted in two different phenotypes: a mutation at position 515 (p.Met515Thr) led to HCM and a mutation at 377 (p.Gly377Ser) caused DCM. These observations confirm genetic overlap in different idiopathic cardiomyopathies and indicate that different mutations in the same gene could lead to different cardiomyopathy phenotypes. The final phenotype may ultimately depend on the (i) location of the mutation in a given protein and (ii) the type of amino acid substitution. For example, mutation in an arginine residue (Arg145) located in the inhibitory domain of *TNNI3* has been found to result in either HCM or RCM depending on the amino acid substitution: Arg145Gly and Arg145Gln have been found to be associated with HCM [10, 11], whereas Arg145Trp is associated with RCM [12].

The published literature shows a wide spectrum of heterogeneity in mutations for different populations; this necessitates the identification of population specific mutations. In the present study, we prospectively investigated 130 cardiomyopathy patients for MYH7 mutations and the influence of these mutations on phenotype and prognosis of the disease. We identified six mutations: three novel and three known mutations in *MYH7* in our cohort. The identification of these new mutations suggests that not all known mutations associated with cardiomyopathy have

been detected and there is need for identification of population specific mutations in different ethnic populations.

These mutations represent dominant negatives by disturbing contractile function despite the production of a normal protein by the remaining normal allele. Consistent with this conclusion is the finding that mutant beta-myosin separated from the heart muscle in cases of hypertrophic cardiomyopathy translocate actin filaments with an abnormally low sliding velocity in motility assays in vitro [13] possibly due to ethnic variations and environmental factors.

We found mutations in *MYH7* in both cardiomyopathy phenotypes, i.e., in HCM and DCM. The association of these mutations with phenotypes ranged from asymmetrical septal hypertrophy to concentric and apical hypertrophy and with both benign and severe forms of the disease, indicating that there is a wide genetic and phenotypic heterogeneity of HCM and DCM in our population similar to that reported for other ethnic populations such as Caucasians and Japanese [3, 5, 11, 14–16].

Traditionally, it has been assumed that HCM and DCM develop during adolescence, to reach full morphologic disease expression at the time of physical maturity (≈ 18 years). The present study, however, suggests that the disease progression may be more complex. The onset of disease varied from second to fifth decade of life in patients with *MYH7* mutations. For example, an HCM proband (H91) had a very early onset of symptoms in the second decade and a proband (H61) was diagnosed with apical hypertrophy in the fifth decade of his life. These observations indicate heterogeneity in genotype and course of the disease.

It has been reported that not all the individuals with a mutation in one of the cardiomyopathy genes will express the clinical features during their lifetime. Our data also indicate that there is considerable variation in penetrance between different mutations within the same gene and even between close relatives in the same family carrying the same specific mutation. With increasing knowledge in molecular genetics and increasing numbers of reported families with different genetic defects, the estimated figures of penetrance might very well change in the future. For the majority of disease causing mutations, the reported numbers of patients are too low to be able to give accurate estimates of penetrance.

The prevalence of *MYH7* mutations were found to be lower in our cohort (HCM 7.2% and 1.6% in DCM) as compared to those observed in other populations [3, 5, 11, 14–16]. This difference may be due the fact that *MYH7* gene mutations may not be predominant in Indians and some other sarcomeric and yet to be identified genes may be involved in the pathophysiology of cardiomyopathy. Our results also suggest that each population may have its own specific mutations.

Conclusion

The present study shows that there is genetic and phenotypic heterogeneity of cardiomyopathies in Indian population. Further, the location and type of mutation in a given sarcomeric gene determines the severity and phenotypic plasticity in cardiomyopathies.

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