Hypertrophic cardiomyopathy (HCM) is a myocardial disorder characterized by unexplained ventricular hypertrophy and myofibrillar disarray and is commonly manifested as dyspnea, palpitations, chest pain and syncope/pre syncope.

It is an autosomal dominant disorder with variable expression and penetrance with a prevalence of about 0.2% in general population[1] and is associated with 16 nuclear and 3 mitochondrial gene mutations. Most of these genes encode for both sarcomeric and non sarcomeric proteins as listed in Table 1. It is further classified into several sub types as asymmetric septal hypertrophy (with and without obstruction), ventricular hypertrophy, hypertrophy of left ventricular posterior wall and apical hypertrophy based on their morphology and clinical presentation.

BACKGROUND: Cardiomyopathies are a heterogeneous group of heart muscle disorders and are classified as 1) Hypertrophic Cardiomyopathy (HCM) 2) Dilated cardiomyopathy (DCM) 3) Restrictive cardiomyopathy (RCM) and 4) Arrhythmogenic right ventricular dysplasia (ARVD) as per WHO classification, of which HCM and DCM are common. HCM is a complex but relatively common form of inherited heart muscle disease with prevalence of 1 in 500 individuals and is commonly associated with sarcomeric gene mutations. Cardiac muscle troponin I (TNNI-3) is one such sarcomeric protein and is a subunit of the thin filament-associated troponin-tropomyosin complex involved in calcium regulation of skeletal and cardiac muscle contraction. Mutations in this gene were found to be associated with a history of sudden cardiac death in HCM patients.

AIM: Therefore the present study aims to identify for mutations associated with troponin I gene in a set of HCM patients from Indian population.

MATERIALS AND METHODS: Mutational analyses of 92 HCM cases were carried out following PCR based SSCP analysis.

RESULTS: The study revealed band pattern variation in 3 cases from a group of 92 HCM patients. This band pattern variation, on sequencing revealed base changes, one at nt 2560 with G>T transversion in exon-5 region with a wobble and others at nt 2479 and nt 2478 with G>C and C>G transversions in the intronic region upstream of the exon 5 on sequencing. Further analysis showed that one of the probands showed apical form of hypertrophy, two others showing asymptomatic septal hypertrophy. Two of these probands showed family history of the condition.

CONCLUSIONS: Hence, the study supports earlier reports of involvement of TNNI-3 in the causation of apical and asymmetrical forms of hypertrophy.

Key words: Genetic variation, hypertrophic cardiomyopathy, sudden cardiac death, troponin-I
One of the sarcomeric genes, Cardiac muscle troponin I (TNNI-3) is a basic globular protein, expressed only in the heart.\[^2\]\ It is a subunit of the thin filament-associated troponin-tropomyosin complex involved in calcium regulation of skeletal and cardiac muscle contraction. Eight coding exons of TNNI-3 code for a polypeptide of 210 amino acids\[^3,4\]\ and mutations in this gene account for a total of 5% HCM cases, with a history of sudden cardiac death and poor prognosis. Hence this gene was screened for mutations in a group of clinically well-characterized HCM patients.

**Materials and Methods**

A total of 92 cases confirmed for HCM based on medical history, physical examination, electrocardiogram and echocardiogram findings were included in the study. These cases were referred to the cardiology units of CARE hospitals, Hyderabad and KEM Hospital, Mumbai. In addition to these cases, 100 voluntary blood donors with no history/family history of any cardiac disorders were included as controls in the study. Informed consent was obtained from all the participating individuals along with Institutional ethics committee clearance.

Isolation of total genomic DNA from whole blood\[^5\]\ of patients and controls was carried out followed by PCR based SSCP analysis.\[^6\]\ Primer sequences for PCR were obtained based on available database of Seidman.\[^7\]\ A PCR mix of final volume 25µl containing 10x PCR buffer (10 mM Tris HCl; 50 mM Kcl), 1.5 mM Mgcl2, 50 p moles of forward and reverse primers, 200 µM dNTPs and 1 U of Taq DNA polymerase was used. All PCR reagents were mixed together in a 0.2 µl PCR tube, which was then placed in a thermal cycler (Eppendorf Master Cycler gradient, Germany) at an annealing temperature of 58°C for 25 cycles.

Amplified products were mixed with equal volumes of formamide loading buffer (formamide 0.9 g/ml, 10 mM NaOH, 11 mM EDTA), denatured at 95°C for 10 min and quenched on ice for 5 min prior to loading. 8 µl of diluted samples were loaded onto 10% non-denaturing polyacrylamide gel and placed in Consortium electrophoresis unit at 150 Volts and 15 mA current. After electrophoresis, band pattern was visualized by silver staining according to the protocol of Orita et al.\[^6\]\ On a stained SSCP gel, mobility shift was recognized as an aberrant band pattern compared to the control sample’s band pattern. PCR products showing an aberrant band pattern on SSCP gels were later purified and commercially sequenced on a 373 DNA analyzer; Macrogen (Korea) for the detection of either SNPs and/or mutations.

**Results and Discussion**

PCR based SSCP analysis [Figure 1A] of 92 HCM cases was carried out on troponin-I gene. Of the 92 individuals screened, 3 probands (FHC 52, 139, 145) revealed a band pattern variation in exon-5. These samples on sequencing revealed heterozygous base changes at nt 2560 with G>T transversion (at codon 68 CGG >CGT) leading for synonymous change for...
Arginine [Figure 1B], with other two base changes at nt 2479 and nt 2478 with G>C and C>G transversions, 26 bases upstream of the exon-5.

The proband (FHC 52) was 38 years old at the time of diagnosis with apical hypertrophic cardiomyopathy, a rare form from the Indian context. Echocardiographic findings showed a thickness of 22mm of interventricular septum and a diameter of 56 mm x 34 mm of left ventricle. Of the 15 family members available, two members had a positive history [Figure 1C]. These two relatives (I-2, II-6) were unavailable for ECG and Echo diagnosis but on physical examination were found to have symptoms of HCM.

FHC 139 (37 years) had obstructive type of cardiomyopathy with LV thickness being 39 mm x 28 mm and 20 mm IVS. When the proband’s family was screened for similar base changes, his daughter was found to have similar band pattern variation and base changes.

Family members of FHC 145 (64 yrs; LV-32 mm x 23 mm; IVS-14 mm) were unavailable for clinical or mutational analysis.

All the three probands showed clinically divergent forms of hypertrophy (FHC 52-Apical form) (FHC-139-Asymmetric septal Hypertrophy (ASH) with obstruction) and (FHC-145-ASH without obstruction) but possessed same set of base changes. This shows that there could be different underlying primary mutations whose expression could have been modified by these changes in exon-5 and intron-4.

Six mutations have been originally described in troponin-I, of which, 5 were missense mutations and one deletion with no disruption of the reading frame.[8] Mutations in troponin I gene have been considered as an infrequent cause of HCM although more mutations have recently been reported.[9,10] Unusual patterns of hypertrophy, including a predominant apical involvement have been associated with troponin I defects.[9,11] Based on the morphologic pattern of disease, troponin I mutations may be more prevalent in populations with a high incidence of apical HCM, as reported in Japan.[8] Therefore the present finding further supports the involvement of multiple base changes in TNNI-3 in the pathogenesis of apical and asymmetrical septal forms of HCM.

Troponin I, a sub unit of troponin complex exerts a modulator influence on the calcium-dependent actin-myosin interaction. The region in which the variations are found in the present study is between the sites interacting with Tn T. Significance of variations in this region in the pathogenesis of the condition needs to be studied by in silico analysis.

Codon bias, the preference for some of the synonymous codons encoding the same amino acid exists in the expression of the genes across organisms. The synonymous changes may exert an effect on the expression by codon-anticodon interaction during translation, which may have effect on translational efficiency.[12] However this needs to be confirmed by functional studies.

Base changes in any of the consensus nucleotides of an intron involved in spliceosome complexes other than the required GT and AG pairs can have a regulatory effect on RNA processing, as reported in beta thalassemia.[13] The base changes observed in present study (26 bases upstream of intron-exon junction) fall near the region involved in secondary lariat structure formation required for splicing. Since the base changes observed in the patients (FHC 52, 139,145) were not found in any of the 100 controls screened, it can be suspected that disruption of spliceosome formation may be involved in disease pathogenesis in these individuals. This needs to be further confirmed by Insilico/invitro studies.

Conclusion

TNNI-3 base changes may also account for rare apical hypertrophy, further strengthening the observations made from other populations. The study shows that the DNA sequence variations found in our population may differ from the changes observed in other populations.

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References

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