Research paper

Phenotypic expressions of a novel mutation in the MYH7 gene responsible for hypertrophic cardiomyopathy in Pune population

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ABSTRACT

Introduction and Aim: Hypertrophic cardiomyopathy (HCM) are the most common kind of cardiac disease that causes left ventricular asymmetric hypertrophy (LVH) or right ventricular hypertrophy (RVH) and non-dilated ventricular chambers. Heterozygous mutation in the MYH7 gene on chromosome 14 causes Left ventricular hypertrophy. The objective of our study was to unravel the mutation spectrum in HCM positive patients and find out novel mutation in MYH7 gene and the impact of these mutations on the pathophysiology of cardiac functioning.

Materials and Methods: Identification and confirmation of HCM patients was done on the basis of the thickness of the Interventricular septum and Left ventricular wall (>13mm ; >15mm). Extraction of DNA from the whole blood of HCM patients and healthy population using the standard phenol- chloroform extraction method was carried out. Polymerase Chain Reaction (PCR) was performed, and samples were processed for Sanger sequencing. Alter the structural arrangement and characteristics of respective mRNAs software's RNA Fold Web server.

Results: We found a novel mutation in the MYH7 gene, which could cause phenotypic expressions similar to that of HCM.

Conclusion: Mutation in the gene may be responsible for causing hypertrophic cardiomyopathy. The mutations reported in our study, are responsible to alter the structural arrangement and characteristics of respective mRNAs.

Keywords: Left ventricular asymmetric hypertrophy; interventricular septum; echocardiography; left ventricular internal diameter.

INTRODUCTION

Clinically stated, left ventricular (LV) hypertrophy without the presence of hypertension or valve disease is referred to as hypertrophic cardiomyopathy (HCM). In the general population, LV hypertrophy without cardiovascular etiology occurs in around 1:500 people (1-3). Alterations to the genes encoding for sarcomeric proteins cause HCM, a familial disease with an autosomal dominant pattern of inheritance. As a result, the LV generally develops in an asymmetrical pattern. HCM is a with a variable clinical history and heterogeneous phenotypic expression, hereditary cardiac disease is marked by sudden mortality as well as debilitating heart failure symptoms (4-5). HCM occurs mostly in the young athletes due to vigorous body exercise and it occurs in the population due to their lifestyle and diet which also play a major role in the phenotypic expression of hypertrophic cardiomyopathy.

There are two types of genes, the causal gene and the modifier gene responsible for causing HCM. Causal genes are mostly related to sarcomeric, nuclear membrane and cytoskeletal genes. In general population HCM is mostly caused by sarcomeric genes like MHY7. TNNT1, TNNT2, MYBPC3 (6). Modifier genes are indirectly responsible for causing HCM. Modifier genes with environmental factors mostly affect the phenotypic expression of HCM (7). Adults with the genotype, including those who die suddenly, may have wall thickness that is normal or almost normal. This is especially true for those who have mutations in MYH7 gene, which typically has mild LVH. Also, be the situation with individuals who have MYH7 mutations, in whom the onset of LVH may begin quite late in life (8).

The major goal of the current investigation was to identify novel mutations in the MYH7 gene and determine how these mutations affected the pathophysiology of cardiac function in HCM positive patients.

METHODOLOGY

Asymmetric septal hypertrophy (ASH) is the hallmark of HCM, but virtually any myocardial section may be affected. To assist in diagnosis, the following 2 D echocardiographic criteria are used:

Inclusion criteria

Echocardiographic criteria for hypertrophic cardiomyopathy interventricular septal thickness -13 mm, the ratio of the thickness of the interventricular septum to the thickness of the left ventricle's posterior wall is 1.5, and the LV wall thickness is 15 mm (or 13 mm in people with a family history of HCM; 9).

Exclusion criteria

Patients having cancer, diabetes, myocardial infarction, renal failure, aortic stenosis, hypertension

(systolic blood pressure ≥140 mmHg and/or diastolic pressure ≥90 mmHg on two or more consecutive visits without anti-hypertensive medications), and coronary artery disease were excluded from the present study (10).

Selection criteria and clinical examination of the patients

In the present study, we had collaborated with the cardiology unit of the Hospital. The research work was authorized by the Institutional Human Ethics Committee, Medical College. Electrocardiographic, echocardiographic, magnetic resonance imaging and physical examinations of patients were performed for the diagnosis of HCM at the Hospital. HCM patients were selected from the hospitals based on 2D echocardiography analysis. Several clinical indicators, including the size of the left auricle, the interventricular septum, the left ventricular internal diameter end diastole (LVIDd), and the left ventricular internal diameter end-systole (LVIDS), and left ventricle ejection fraction (LVEF) were also considered for the study. For the examination of echocardiographic parameters, the reference values given by Prajapati *et al*., (11) was followed. The clinical data from 74 HCM patients between the age group 20 to 80 (mean age ±37.44 years) and 40 non-HCM healthy persons (mean age ±30.42 years) was collected from Hospital and was investigated for clinical characteristics. IVS more than 13 mm was considered as a characteristic of HCM (12).

Statistical analysis

Statistical analyses were carried by Past 3.02a software. For non-parametric data, Kruskal-Wallis tests were employed for comparison of sample mean, and a post hoc test by Mann- Whitney. Graphical representation was performed in GraphPad Prism version 8.0.0, $p<0.05$ were considered significant for the current study.

Blood collection

Before data and blood sample collection, written informed consent was taken from the patients. 5 ml of blood was collected from the cardiology unit at Bharati Hospital. Blood samples were carried to the Research Institute for further analysis.

Genomic DNA isolation

Using the phenol-chloroform extraction technique, genomic DNA was extracted from whole blood (13,14). 40 HCM and two control samples were selected between the age group 20 to 50 years for the screening MYH7 gene.

Primer designing and polymerase chain reaction (PCR)

Primers were designed on the NCBI platform for single nucleotide variants with possible clinical effects. Primers were designed only to amplify the targeted region (Table 1). The Veriti 96 Well Thermal Cycler was used to conduct the PCR (AB Applied Biosystems) separately for different variants of the MYH7 gene in HCM patients. To check the amplification of the PCR products agarose gel electrophoresis was performed and the gel was observed under Gel Dock (Cell Biosciences, Alpha Imager HP, H6Z0812M).

Restriction digestion

Primers were designed on NEB cutters by using the amplified DNA Fasta sequence. PCR products larger than 300 bp were by restriction enzymes by diluting molecular grade water, 2 ul of buffer, and 1 ul of restriction enzyme were added, (3 times that of S/V) on 2% agarose gel, digested products were seen and recorded.

Polyacrylamide gel electrophoresis

The PCR mixture was added with 20 l of phenol, chloroform, and isoamyl alcohol, vortexed for 30 seconds, then centrifuged for two minutes at 10,000 rpm at room temperature. A new tube was used to aspirate and transfer the upper watery layer. After adding 10 ul of formamide and pipetting back and forth to thoroughly combine all the components, the sample was denatured at 95ºC for 10 min. Immediately samples were snap-chilled for 10 minutes. Non-denaturing polyacrylamide gel electrophoresis (PAGE) was performed on the samples, were run on 8 to 10% non-denaturing PAGE at 4ºC for 10-12 hours. Detection of mutation in HCM patients was done by single strand conformation polymorphism (SSCP) techniques (Fig. 1).

Silver nitrate staining

Polyacrylamide gel was further proceeded for silver nitrate staining, silver staining was done as per the method of Bredt and Solomon (15), to observe the band shift in targeted DNA sequence digested by restriction enzyme. Photography was ready by digital camera for future records.

Sanger sequencing

The amplified PCR product was purified by a PCR purification kit (Thermo Scientific GeneJET). The Sanger sequencing of the target portion was done for a gene of HCM and control samples. Samples were visualized on 2% agarose gels and processed for bi-directional Sanger sequencing.

***In silico* analysis**

Chromatograms were seen using Finch TV 1.4.0 and bases with a quality value of >20 underwent additional processing. The data from the ExAC browser beta, 1000 genomes browser, and dbSNP were cross-checked to see if the difference was classified as 'new' in status. The PSIPRED tool predicted secondary protein structures. The

PolyPhen-2 tool was utilized to validate the effect of missense mutations on protein functioning. Human Splicing Finder (HSF3.1) was employed to determine how differences affected splicing.

Analysis of variants

With the aid of various bioinformatics tools (GWAS, SNPdb, and Gene browsers), exonic non-synonymous, splice-site, and loss of function variants were examined. The classification of novel variations was based on the combination of the predictions made by the three forms of prediction software (POLYPHEN, SIFT, and PROVEAN): 'likely pathogenic,' 'likely benign,' and 'uncertain importance".

RESULTS

Study of echocardiographic parameters

The Left ventricular wall thickness and interventricular septum size were significantly increased in HCM patients, while there is no significant difference in ejection fraction and Aortic root as compared to control samples. The size of the left atrium in hypertrophic patients is comparatively enlarged than those of control participants but is within the reference range. The Left ventricular posterior wall dimension was also found to be significantly increased in HCM patients than in control. The size of the Left ventricular internal diameter end diastole (LVIDd) in HCM patients was remarkably enlarged, whereas the size of the Left ventricular internal diameter end-systole (LVIDs) was slightly reduced compared to a healthy person. The 21 to 60 years age group was selected to study the HCM in table 1.

Table 1: Evaluation of 2D echo reports of HCM in Pune population

Echo parameters	HCM (n=45) ♂	HCM (n=29)♀	Control (n=40)	Reference range	p-Value
Age	47.73	48.86	49.07	-	-
LVWT	25.62	22.68	11.07	11-13 mm	0.0001
Aortic root	24.93	24.89	24.97	23-43mm	0.95
Left atrium	38.6	36.86	31.77	19-40 mm	0.0001
IVS	17.53	16.65	9.15	6-11mm	0.0001
LVID-D	46.77	46.79	44.5	35-56mm	0.01
LVID-S	25.15	25.31	27.55	20-40mm	0.0003
LVPWD	15.71	14.79	9.35	6-11mm	0.0001
Ejection Fraction (%)	56	56.20	56	55-70 %	1

LVWT-Left ventricle Wall Thickness, IVS interventricular septum, LVID-D Left ventricular internal diameter end diastole, LVID-S Left ventricular internal diameter end systole, LVPWD Left ventricular posterior wall dimension, reference values taken from (16,17).

Polyacrylamide gel electrophoresis and staining

PAGE was further proceeded for silver nitrate staining to check the change in band shift in hypertrophic cardiomyopathy patients in Pune population. We observed the changes in the position of bands in MYH7 genes in HCM patients in Pune population.

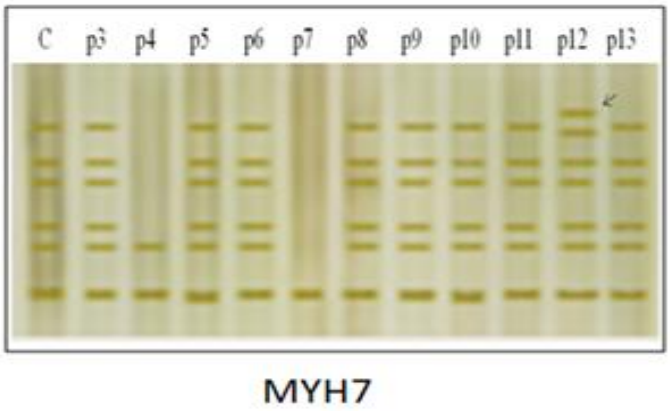

Fig. 1: Polyacrylamide gel electrophoresis for MYH7 gene stained with silver nitrate. The arrow indicates band shift in proband 12.

Sanger sequencing

Sanger sequencing was done for the confirmation of variants identified by Polyacrylamide gel electrophoresis and stained by silver nitrate staining technique in the MYH7 genes, we found change in the nucleotides sequence at functional positions of genes which affects the mRNA and protein secondary structure (Fig. 2).

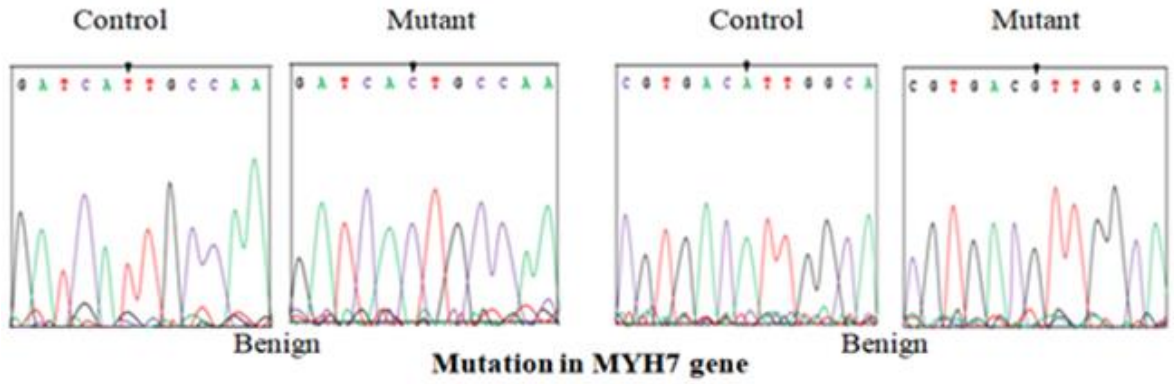

Fig. 2: Electro-chromatogram showing mutation in genes in hypertrophic cardiomyopathy in Pune population. The arrow indicates the change in nucleotide sequence.

Analysis of mutations

Mutations were analyzed by Polyphene and mutation taster bioinformatics tools (Fig. 3). We observed that replacement of nucleotides alters the amino acids, which affects the secondary protein structure, which could be damaging can cause HCM in patients and can affect the function of protein, benign could not affect the function of protein in fig. 4.

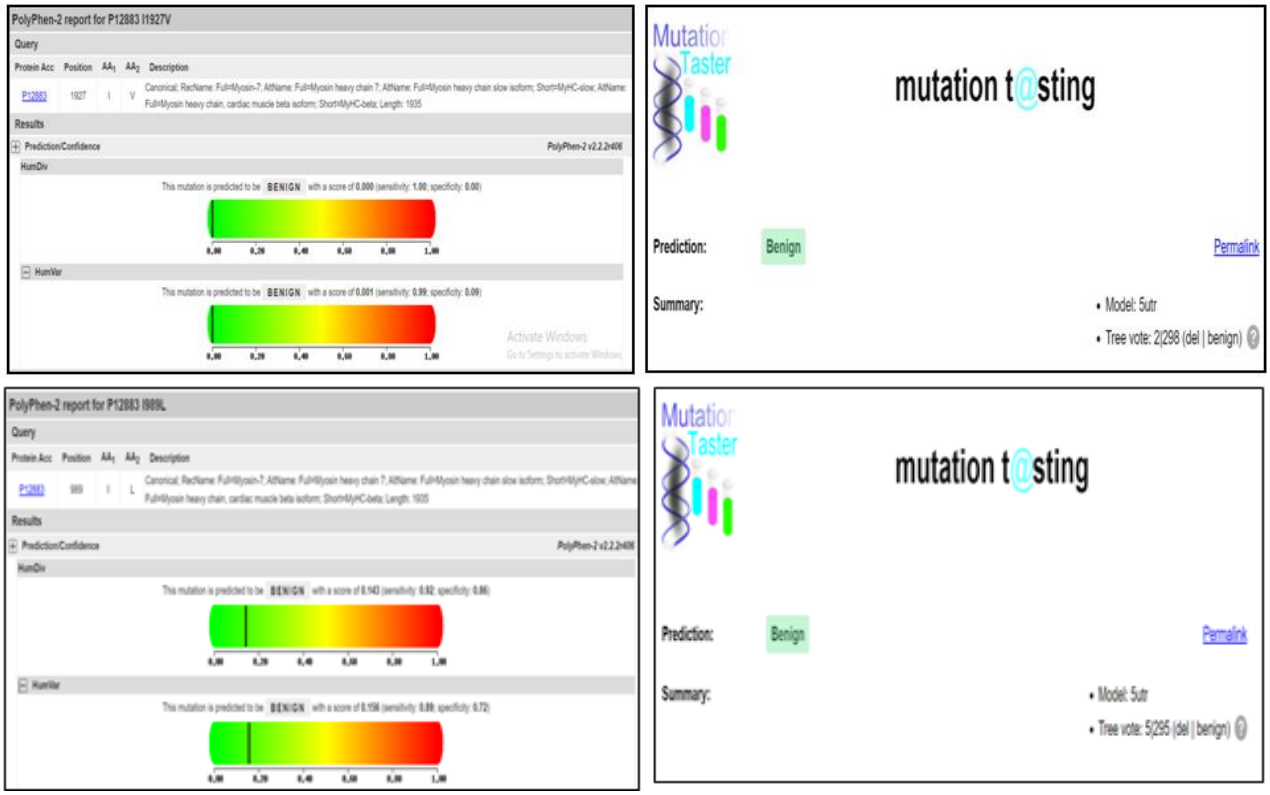

Fig. 3: i) Analysis of novel mutation ii) Analysis of reported mutation in MYH7 gene by Polyphene 2 and mutation tasting tools.

Fig. 4: These are secondary structures of protein, there is replacement of amino acid Isoleucine replaced by valine, the arrow indicates the changes seen in the structure of protein, changes were observed in the folding pattern of protein.

DISCUSSION

In the present study the article describes the results of clinical parameters, echocardiographic characteristics and the genetic screening obtained through Sanger sequencing approach in the Pune population possibly well characterized HCM cases, distributed into two groups wild type and diseased caused population. The main aspect of our study was to investigate echocardiographic characters and find out the mutation in genes related to hypertrophic cardiomyopathy in Pune population. In an echocardiographic characteristic study we found, there was a significant difference in all the echo parameters except aortic root and ejection fraction. Further the left ventricular wall thickness and interventricular septum size were significantly increased in HCM patients (p-0.0001) (18). The normal range for LVWT is 11 to 13 mm, if the thickness more than 13mm, is considered as hypertrophy, all subjects selected for study were having thickness mean value 25.62 mm in males and 22.68 mm in females, mean value for LVWT in control was 11.07mm. In our study all the patients have increased left ventricular wall thickness meaning the same results were observed in Debonnaire *et al.*, and Ozava *et al.*, (19,20). There is no significant difference in ejection fraction and aortic root and the similar results were observed in Kubler *et al.*, (21), the mean value for EF in hypertrophied male, female and control was 56 percent which was within normal range (20). The aortic root mean values for all three groups were nearly similar within reference [23-43 mm]. The size of left atrium is enlarged in hypertrophic patients both in male and female compared to normal, and the similar results were seen in Singh *et al.*, (22). But within the reference range [19-40 mm], the enlarged size of left atrium in HCM patient was near to 38 and 36 mm respectively (20), and the size for left atrium in healthy group was 31.77mm. The size of interventricular septum was thickened in both genders in mutant, the mean value was near to 17mm, while IVS was normal in wild type, mean value 9.15 mm, the size of interventricular septal thickness increased in hypertrophic cardiomyopathy patients and same characters were seen in Doi *et al.*, (20,23). The left ventricular posterior wall dimension was also expanded 5-6 mm in hypertrophic patients than control [p-0.0001], the mean value for control was 9.35 mm, similar types of results were obtained by Spirito *et al.*, (24). The size of left ventricular internal diameter end diastole (LVIDd) was slightly enlarged by 2mm (p-0.01) while the size of left ventricular internal diameter end systole (LVIDs) was slightly reduced by 2mm compared to healthy persons and the similar condition was observed by Sanderson *et al.*, (20,25).

Further in this study, of all HCM patients' maximum percentage of mitral regurgitation (24%), aortic regurgitation (24%), and grade I diastolic dysfunction (30%) was observed. On sequencing all exons of MYH7 genes, we found a total 6 mutations in which we neglected intronic variants and considered only exonic variants for the study. We considered only exonic variance in which one mutation was novel and another was reported benign mutation. We tested these variances on different bioinformatics tools like POLYPHEN, SIFT, and PROVEAN.

CONCLUSION

This study discovered the I1927V mutations of the *MYH7* gene. Mutations found in MYH7 gene are benign because aliphatic amino acids are replaced by aliphatic amino acids. Alteration was observed in mRNA and Secondary protein structure. Up to 35 percent of all cases of familial hypertrophic cardiomyopathy are caused by mutations in the MYH7 gene. The heart muscle thickens (hypertrophies) in response to this situation. All those with familial hypertrophic cardiomyopathy have a higher chance of heart failure and untimely death, even if some do not appear to have any visible health symptoms.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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