Original Article

MUTATIONAL ANALYSIS OF MYBPC3 GENE IN DILATED CARDIOMYOPATHY PATIENTS IN NORTH INDIAN POPULATION

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ABSTRACT

Introduction: Today, molecular cardiology is characterized by the integration of high-technology laboratory studies and clinical medicine. Molecular genetics has redefined the etiology and diagnostic criteria for numerous diseases and has led to the development of new, individualized treatment regimens for several cardiovascular diseases. Amongst all, dilated cardiomyopathy is the commonest cause of heart failure. This study was conducted to identify the possible genetic change in dilated cardiomyopathy in North Indian population.

Material & Methods: Blood samples of dilated cardiomyopathy patients were collected from Cardiology OPD, Sir Sunderlal Hospital, Banaras Hindu University. DNA was isolated using salting out method. PCR was done to amplify exons 32, 33 and 34 of MYBPC3 gene. The PCR product was sequenced to detect the mutational changes in Exons 32-34 of MYBPC3 gene.

Results: There were 65 control samples and 65 DCM samples were collected. Total 76 intronic variations were reported. In three (BHU/15/425, BHU/15/450, BHU/16/89) patients, disease causing pathogenic variant c3624_3625insC (rs397516029, HMGCD00910028) was reported in MYBPC3 gene. Insertion of G at 47354119_47354120 position was reported that lead to a frameshift mutation in three subjects. Several Missence variants were also reported 47354121G>C, 47353899C>T, 47353715C>A, 47353647A>C, 47353626G>T that are present in coding region and may lead to alteration in protein structure and function.

Conclusion: Evidence from previous study reported that MYBPC3 play important role in cardiac contraction and responsible for pathogenesis of dilated cardiomyopathy. Therefore, the identification of frequent genetic transmission of dilated cardiomyopathy provides an important tool for the study of pathogenesis of this disease, which is a frequent cause of admission to the hospital and of heart failure.

Keywords: Dilated cardiomyopathy, myosin binding protein C, sequencing, DNA.

INTRODUCTION

Disorders of the heart leading to heart failure are leading cause of morbidity and mortality. Since the term “cardiomyopathy” was coined 30 years ago to describe a group of myocardial diseases of unknown cause, which was termed as idiopathic cardiomyopathy [1].

Out of all types of cardiomyopathies, dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) are the two major cardiomyopathies (Fig. 1). Other clinical cardiomyopathies include restrictive cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy [2].

DCM is defined by the presence of: a) fractional shortening (FS) less than 25% (> 2SD) and/or ejection fraction less than 45% (> 2SD); and b) left ventricular end diastolic diameter (LVEDD) greater than 117% (>2SD of the predicted value of 112% corrected for age and body surface area, BSA) [3] excluding any known cause of myocardial disease. In the context of a familial DCM, these criteria are used to diagnose the
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Recent studies report that 20-35% cases of dilated cardiomyopathy are considered to be familial. Therefore, it is important to study the genetic factors responsible for DCM. Many genes are responsible for DCM out of which the most common are MYBPC3, MYH7, TNNT2, TTN, SGCD. In this study we screened familial DCM patients for the presence of mutation in MYBPC3 gene by DNA isolation and by DNA sequencing [5].

MYBPC 3 (Myosin Binding Protein C) Gene
The MYBPC3 gene provides instructions for making the cardiac myosin binding protein C (cardiac MYBP-C), which contains 35 exons and is found in heart (cardiac) muscle cells. In these cells, cardiac MYBP-C is associated with a structure called the sarcomere, which is the basic unit of muscle contraction. Sarcomeres are made up of thick and thin filaments. The overlapping thick and thin filaments attach to each other and release, which allows the filaments to move relative to one another so that muscles can contract. Regular contractions of cardiac muscle pump blood to the rest of the body [6].

In cardiac muscle sarcomeres, cardiac MYBP-C attaches to thick filaments and keeps them from being broken down. Cardiac MYBP-C has chemical groups called phosphate groups attached to it; when the phosphate groups are removed; cardiac MYBP-C is broken down, followed by the breakdown of the proteins of the thick filament. Cardiac MYBP-C also regulates the rate of muscle contraction, although the mechanism is not fully understood [7].

Chromosomal Location
Cytogenetic location: 11p11.2, which is the short (p) arm of chromosome 11 at position 11.2 (Fig. 2).

Molecular location: base pairs 47,331,406 to 47,352,702 on chromosome 11 (Homo-sapiens Annotation Release 108, GRCh38.p7) [7].

Fig. 1: Morphological changes to the heart in cardiomyopathy. (A) Normal heart. (B) In DCM, the heart enlarges with increased diameter and reduced function. (C) In HCM, the myocardium especially in the LV becomes thickened, leading to impaired filling and emptying [4].

Fig. 2: Cytogenetic location of MYBPC3 gene
The main objective of our study was to evaluate mutational changes in genes MYBPC3 responsible or causing DCM as well as HCM in the population of North Indians from Eastern Uttar Pradesh region.

MATERIAL AND METHODS

Sample Collection
3 to 5 ml of peripheral blood was collected in EDTA coated vials after taking informed consent from patients of dilated cardiomyopathy, samples were collected from the Outpatient Department of Cardiology, Sir Sunderlal Hospital of Institute of Medical Sciences, Banaras Hindu University, Varanasi (from 2015-2017). Echocardiogram (ECG) and clinical history of patient were noted; ischemic dilated cardiomyopathy patients were excluded from this study. The study protocol was approved by Ethical Committee of SS hospital, BHU, Varanasi.

DNA Extraction and PCR Amplification
DNA isolation was done by “Salting out method” and dissolved in TE buffer. Patient samples can be readily checked for concentration and quality using the Nano Drop 1000 Spectrophotometer. After DNA extraction coding region of exons 32-34 were amplified by using following primers exon 32-34F 5’ GGCTCAGCCACTGACTTGT 3’ and 32-34R 5’ AGGGGCCTAGCTTTGTGTG 3’ after amplification 5 µl PCR amplified products were loaded with DNA loading dye to check the amplification.

DNA Sequencing
When PCR amplification was complete, PCR products was purified with Exo Sap reagent. The purified PCR products were then sequenced using the sequencing kit and sequencer from Applied Biosystems, USA. Sequencing reactions were analysed using 3130xL Genetic Analyzer (Applied Biosystem R). Sequencing files were obtained from the 3130xL Genetic Analyzer (applied Biosystems R) were analysed using FinchTV.
viewer. Further analysis was done using MEGA 6 software and Mutation Taster software.

**OBSERVATIONS AND RESULTS**

Total of 65 patients samples were collected out of which 50 were male and 15 were female. Agarose gel electrophoresis results (Fig. 3-5) and sequencing results (Fig. 6,7) exhibited various synonymous and non-synonymous mutations (Table 1). A disease causing pathogenic variation was found in one of the patient.

**Fig. 3: 2% Agarose Gel Electrophoresis for MYBPC3 having amplicon size 953 bp**

**Fig. 4: 2% Agarose Gel Electrophoresis for MYBPC3 having amplicon size 953 bp**

**Fig. 5: 2% Agarose Gel Electrophoresis for MYBPC3 having amplicon size 953 bp**

**Fig. 6: Representative sequence electropherograms showing c.3624_3625insC substitution in patient BHU/15/450 in MYBPC3 gene**

**Fig. 7: Multiple sequence alignment of MYBPC 3 gene in patient BHU/15/450 with control sample.**
### Table 1: Showing various synonymous and non-synonymous mutations

| S. No. | Genomic position | Patient ID | Aminoacid change | Mutation taster |
|--------|------------------|------------|------------------|-----------------|
| **MYBPC3** |                 |            |                  |                 |
| 1      | Chr 11: 47354160C>A | BHU/15/407 | G1195V           | rs730880595     |
| 2      | Chr 11: 47354127>47354128 ins C | BHU/15/408 | S1207            | HMGD CM057198, CM068014 |
| 3      | Chr 11: 47354121G>C | BHU/15/408 | P1208R           | SINGLE BASE EXCHANGE,CDS |
| 4      | Chr 11: 47354072_47354077 ins C | BHU/15/408 |                  | INTRON VARIANT  |
| 5      | Chr 11: 47354038_47354059 ins G | BHU/15/408 |                  | INTRON VARIANT  |
| 6      | Chr 11: 47353899C>T | BHU/15/408 | SINGLE BASE EXCHANGE | rs11039186     |
| 7      | Chr 11: 47353536_47353537 ins A | BHU/15/408 |                  | INTRON VARIANT  |
| 8      | Chr 11: 47353509_47353510 ins A | BHU/15/408 |                  | INTRON VARIANT  |
| 9      | Chr 11: 47353464_47353465 ins A | BHU/15/408 |                  | INTRON VARIANT  |
| 10     | Chr 11: 47353715C>A | BHU/15/423 | R1241I           | CDS             |
| 11     | Chr 11: 47353637A>G | BHU/15/423 | C1264G           | CDS             |
| 12     | Chr 11: 47353626G>T | BHU/15/423 |                  | HGMD CM086866   |
| 13     | Chr 11: 47354032A>T | BHU/15/424 |                  | INTRON VARIANT  |
| 14     | Chr 11: 47354092C>A | BHU/15/424 |                  | INTRON VARIANT  |
| 15     | Chr 11: 47354029_47354030 ins A | BHU/15/425 |                  | INTRON VARIANT  |
| 16     | Chr 11: 47353476_47353477 ins C | BHU/15/424 |                  | INTRON VARIANT  |
| 17     | Chr 11: 47353448A>G | BHU/15/424 |                  | INTRON VARIANT  |
| 18     | Chr 11: 47353532T>A | BHU/15/424 |                  | INTRON VARIANT  |
| 19     | Chr 11: 47353502A>T | BHU/15/424 |                  | INTRON VARIANT  |
| 20     | Chr 11: 47353496G>C | BHU/15/424 |                  | INTRON VARIANT  |
| 21     | Chr 11: 47353436_47353437 ins A | BHU/15/424 |                  | INTRON VARIANT  |
| 22     | Chr 11: 47353453T>C | BHU/15/424 |                  | INTRON VARIANT  |
| 23     | Chr 11: 47354119_47354120 ins G | BHU/15/425 | K1209Q           | rs397516029 FRAMESHIFT |
| 24     | Chr 11: 47354095_47354096 ins C | BHU/15/425 |                  | INTRON VARIANT  |
| 25     | Chr 11: 47354068G>A | BHU/15/425 |                  | INTRON VARIANT  | rs3729802     |
| 26     | Chr 11: 47354040G>T | BHU/15/425 |                  | INTRON VARIANT  |
| 27     | Chr 11: 47353498G>A | BHU/15/425 |                  | INTRON VARIANT  | rs2290146     |
| 28     | Chr 11: 47354119_47354120 ins G | BHU/15/450 | K1209Q           | rs397516029     |
| S. No. | Genomic position | Patient ID | Aminoacid change | Mutation taster |
|-------|------------------|------------|------------------|-----------------|
| 29    | Chr 11: 47354068G>A | BHU/15/450 |                  | INTRON VARIANT  |
| 30    | Chr 11: 47353536_47353537 insA | BHU/15/450 |                  | INTRON VARIANT  |
| 31    | Chr 11: 47353498G>A | BHU/15/450 |                  | INTRON VARIANT  |
| 32    | Chr 11: 47353464_47353465 insA | BHU/15/450 |                  | INTRON VARIANT  |
| 33    | Chr 11: 47353434_47353435 insA | BHU/15/450 |                  | INTRON VARIANT  |
| 34    | Chr 11: 47353464_47353465 insA | BHU/15/464 |                  | INTRON VARIANT  |
| 35    | Chr 11: 47354090 delC | BHU/15/465 |                  | INTRON VARIANT  |
| 36    | Chr 11: 47353502C>A | BHU/15/465 |                  | INTRON VARIANT  |
| 37    | Chr 11: 47353464_47353465 insA | BHU/15/465 |                  | INTRON VARIANT  |
| 38    | Chr 11: 47353453_47353454 insA | BHU/15/465 |                  | INTRON VARIANT  |
| 39    | Chr 11: 473534090delC | BHU/15/465 |                  | INTRON VARIANT  |
| 40    | Chr 11: 473534066C>T | BHU/15/465 |                  | INTRON VARIANT  |
| 41    | Chr 11: 47353536_47353537 insA | BHU/15/608 |                  | INTRON VARIANT  |
| 42    | Chr 11: 47353485_47353486 insA | BHU/15/608 |                  | INTRON VARIANT  |
| 43    | Chr 11: 47353464_47353465 insA | BHU/15/608 |                  | INTRON VARIANT  |
| 44    | Chr 11: 47353457-47353458 insA | BHU/15/608 |                  | INTRON VARIANT  |
| 45    | Chr 11: 47353745C>T | BHU/16/08  | S1231N           | HGMD CI014153   |
| 46    | Chr 11: 47353474C>T | BHU/16/08  |                  | CDS             |
| 47    | Chr 11: 47353496G>C | BHU/16/08  |                  | INTRON VARIANT  |
| 48    | Chr 11: 47354093delG | BHU/16/39  |                  | INTRON VARIANT  |
| 49    | Chr 11: 47354085G>A | BHU/16/39  |                  | INTRON VARIANT  |
| 50    | Chr 11: 47353899C>T | BHU/16/39  | rs11039186       | INTRON VARIANT  |
| 51    | Chr 11: 47353434_47353435 insA | BHU/16/39  |                  | INTRON VARIANT  |
| 52    | Chr 11: 473534038_47354039 insG | BHU/16/58  |                  | INTRON VARIANT  |
| 53    | Chr 11: 47353536_47353537 insG | BHU/16/58  |                  | INTRON VARIANT  |
| 54    | Chr 11: 47353498G>A | BHU/16/58  | rs2290146        | INTRON VARIANT  |
| 55    | Chr 11: 47353464_47353465 insA | BHU/16/58  |                  | INTRON VARIANT  |
| 56    | Chr 11: 47353874A>T | BHU/16/58  |                  | INTRON VARIANT  |
| 57    | Chr 11: 47353864G>C | BHU/16/58  |                  | INTRON VARIANT  |
| 58    | Chr 11: 47353756G>A | BHU/16/58  |                  | CDS             |
| 59    | Chr 11: 47353459_47353460 insA | BHU/16/58  |                  | INTRON VARIANT  |
| 60    | Chr 11: 47353451_47353452 insC | BHU/16/58  |                  | INTRON VARIANT  |
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| S. No. | Genomic position | Patient ID | Aminoacid change | Mutation taster |
|--------|------------------|------------|------------------|-----------------|
| 61     | Chr 11:47354093delG | BHU/16/59 | INTRON VARIANT   |                 |
| 62     | Chr 11: 47354090C>G | BHU/16/59 | INTRON VARIANT   |                 |
| 63     | Chr 11: 47353899C>T | BHU/16/59 | rs11039186        |                 |
| 64     | Chr 11: 47353457_47353458 insA | BHU/16/59 | INTRON VARIANT   |                 |
| 65     | Chr 11: 47353899C>T | BHU/16/59 | rs11039186        |                 |
| 66     | Chr 11: 47354090delLC | BHU/16/70 | INTRON VARIANT   |                 |
| 67     | Chr 11: 47354068G>A | BHU/16/70 | rs3729802         |                 |
| 68     | Chr 11: 47353498G>A | BHU/16/70 | rs2290146         |                 |
| 69     | Chr 11: 47353457_47353458 insA | BHU/16/70 | INTRON VARIANT   |                 |
| 70     | Chr 11: 47353434_47353435 insA | BHU/16/70 | INTRON VARIANT   |                 |
| 71     | Chr11:47354119_47354120 insG | BHU/16/89 | K1209Q           | rs397516029     |
| 72     | Chr11:47354090_47354090 delC | BHU/16/89 | INTRON VARIANT   |                 |
| 73     | Chr11:47354068G>A | BHU/16/89 | rs3729802         |                 |
| 74     | Chr11:47353498G>A | BHU/16/89 | INTRON VARIANT   | rs2290146       |
| 75     | Chr11:47353434_47353435insA | BHU/16/89 | INTRON VARIANT   |                 |

**DISCUSSION**

Dilated cardiomyopathy is most commonly associated with various other heart disorders. The most common associated disorder is congestive heart failure. In DCM there is ventricular chamber enlargement, the ventricular walls become thin and there is depressed left ventricular systolic function. It is most commonly idiopathic or familial. There may be various modes of inheritance i.e. autosomal dominant, autosomal recessive, X-linked or mitochondrial mode of inheritance.

Present study combined with previous studies, provides extensive data of association with the genetic factors causing DCM. We used Sanger based sequencing which is still the gold standard for sequencing sensitivity and specificity. In present study **Exon 32-34 of MYBPC 3** gene was sequenced to identify possible genetic cause of dilated cardiomyopathy. Mutations in the gene for cardiac myosin-binding protein C account for main genetic cause of maximum cases of familial hypertrophic cardiomyopathy and dilated cardiomyopathy. In present study various synonymous and non-synonymous variations had been observed.

In MYBPC3 gene, several intronic variations observed. Total 76 intronic variations were reported. Intrinsic variation does not alter protein structure but it may change splice site that may affect the protein structure and function. In three (BHU/15/425, BHU/15/450, BHU/16/89) patients disease causing variant c3624_3625insC (rs397516029, HMGD CD0910628) was reported in MYBPC3 gene. Same variation was reported by Hershberger et al., 2010 [8].

Some novel variant reported may lead to DCM. Insertion of G at 47354119_47354120 position reported lead to a frameshift mutation in three subjects. Several missence variants were also observed 47354121G>C, 47353899C>T, 47353715C>A, 47353647A>C, 47353626G>T that are present in coding region and may lead to alteration in protein structure and function. Because of these variations are present in C terminal domain of protein that play very important role.
Cardiac myosin-binding protein C (MYBPC3) is arrayed transversely in sarcomere A-bands and binds myosin heavy chain in thick filaments and in elastic filaments. Phosphorylation of this protein appears to modulate contraction [9]. The prognosis in patients with dilated cardiomyopathy is considered to be poor as morbidity and mortality rates are very high. Moreover, DCM is the chief indication for heart transplantation. So, it is important to diagnose the disease early for better treatment.

Evidence from previous study reported that MYBPC3 play important role in cardiac contraction and responsible for pathogenesis of dilated cardiomyopathy [10].

CONCLUSION

The identification of frequent genetic transmission of dilated cardiomyopathy provides an important tool for the study of pathogenesis of this disease, which is a frequent cause of admission to the hospital and of heart failure. Molecular genetic techniques help to identify the gene causing familial dilated cardiomyopathy and can be used to study the effects of altered gene product whether it is frameshift mutation or missense variation. Whereas early features of dilated cardiomyopathy can be identified by echocardiography or electrocardiogram or magnetic resonance imaging. The genetic as well as imaging information together provides accurate status of disease which helps in the management and prognosis of dilated cardiomyopathy. As soon as the genetic cause of dilated cardiomyopathy is diagnosed, the patient should undergo genetic counselling. It includes family history, education regarding transmission, benefits of regular cardiac screening tests. Multidisciplinary medical care for DCM includes genetic counsellors, cardiologists, medical geneticists.

In present study, mutational analysis of 32-34 exons of MYBPC3 gene and 14-15 exon of TNNT2 gene had been done. Various synonymous and non-synonymous variations had been reported. Several intronic variation, frameshift mutation and missense variation are reported in both genes. That suggest these variation may be responsible for pathogenesis of dilated cardiomyopathy in patients of North Indian population. So, all families having familial transmission of dilated cardiomyopathy must have essential knowledge regarding the disease.

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