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 MYBPC 3 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, HMGD CD0910628) 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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proband in a family.

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]

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. 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 5µl PCR amplified products were loaded with DNA loading dye to check the amplification.

DNA Sequencing

When PCR amplification was complete, PCR products were 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

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	Chr11: 47354121G>C	BHU/15/408	P1208R	SINGLE BASE EXCHANGE,CDS
4	Chr 11: 47354072_4735407 ins C	BHU/15/408		INTRON VARIANT
5	Chr 11:47354038_47354039 ins G	BHU/15/408		INTRON VARIANT
6	Chr 11: 47353899C>T	BHU/15/408	SINGLE BASE EXCHANGE	rs11039186
7	Chr 11: 47353536_47353537 insA	BHU/15/408		INTRON VARIANT
8	Chr 11: 47353509_47353510 insA	BHU/15/408		INTRON VARIANT
9	Chr 11: 47353464_47353465 insA	BHU/15/408		INTRON VARIANT
10	Chr 11: 47353715C>A	BHU/15/423	R1241I	CDS
11	Chr 11: 47353647A>C	BHU/15/423	C1264G	CDS
12	Chr 11: 47353626G>T	BHU/15/423	CDS	HGMD CM086866
13	Chr 11: 47354102C>A	BHU/15/424		INTRON VARIANT
14	Chr 11: 47354090C>T	BHU/15/424		INTRON VARIANT
15	Chr 11: 47354019_47354020 insA	BHU/15/424		INTRON VARIANT
16	Chr 11: 47353977_47353978 insC	BHU/15/424		INTRON VARIANT
17	Chr 11: 47353948A>C	BHU/15/424		INTRON VARIANT
18	Chr 11: 47353536T>A	BHU/15/424		INTRON VARIANT
19	Chr 11: 47353502>A	BHU/15/424		INTRON VARIANT
20	Chr 11: 47353496G>C	BHU/15/424		INTRON VARIANT
21	Chr 11:47353464_47353465 insA	BHU/15/424		INTRON VARIANT
22	Chr 11: 47353453T>C	BHU/15/424		INTRON VARIANT
23	Chr 11: 47354119_47354120 insG	BHU/15/425	K1209Q	rs397516029 FRAMESHIFT
24	Chr 11: 47354095_47354096 insC	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 insG	BHU/15/450	K1209Q	rs397516029

S. No.	Genomic position	Patient ID	Aminoacid change	Mutation taster
				FRAMESHIFT
29	Chr 11: 47354068G>A	BHU/15/450		INTRON VARIANT rs3729802
30	Chr 11: 47353536_47353537 insA	BHU/15/450		INTRON VARIANT
31	Chr 11: 47353498G>A	BHU/15/450		INTRON VARIANT rs2290146
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_47353465insA	BHU/15/465		INTRON VARIANT
38	Chr 11: 47353453_4735345 insT	BHU/15/465		INTRON VARIANT
39	Chr 11: 47354090delC	BHU/15/608		INTRON VARIANT
40	Chr 11: 47354065C>T	BHU/15/608		INTRON VARIANT
41	Chr 11: 47353536_47353537insA	BHU/15/608		INTRON VARIANT
42	Chr 11: 47353485_47353486 insA	BHU/15/608		INTRON VARIANT
43	Chr 11: 47353464_47353465insA	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: 47353741C>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
51	Chr 11: 47353434_47353435insA	BHU/16/39		INTRON VARIANT
52	Chr 11: 47354038_47354039 insG	BHU/16/58		INTRON VARIANT
53	Chr 11: 47353536_47353537 insA	BHU/16/58		INTRON VARIANT
54	Chr 11: 47353498G>A	BHU/16/58		rs2290146
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

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: 47354090delC	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	Chr11:47353418_47353419 insT	BHU/16/70		INTRON VARIANT
72	Chr11:47354119_47354120 insG	BHU/16/89	K1209Q	rs397516029 FRAMESHIFT PRESENT
73	Chr11:47354090_47354090 delC	BHU/16/89		INTRON VARIANT
74	Chr11:47354068G>A	BHU/16/89		rs3729802
75	Chr11:47353498G>A	BHU/16/89		INTRON VARIANT rs2290146
76	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. Intronic 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 missense 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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