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ORIGINAL RESEARCH

Novel *MYBPC3* Mutations in Indian Population with Cardiomyopathies

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Background: Mutations in Myosin Binding Protein C (*MYBPC3*) are one of the most frequent causes of cardiomyopathies in the world, but not much data are available in India.

Methods: We carried out targeted direct sequencing of *MYBPC3* in 115 hypertrophic (HCM) and 127 dilated (DCM) cardiomyopathies against 197 ethnically matched healthy controls from India.

Results: We detected 34 single nucleotide variations in *MYBPC3*, of which 19 were novel. We found a splice site mutation [(IVS6 +2T) T>G] and 16 missense mutations in Indian cardiomyopathies [5 in HCM; E258K, T262S, H287L, R408M, V483A: 4 in DCM; T146N, V321L, A392T, E393K and 7 in both HCM and DCM; L104M, V158M, S236G, R272C, T290A, G522E, A626V], but those were absent in 197 normal healthy controls. Interestingly, we found 7 out of 16 missense mutations (V158M, E258K, R272C, A392T, V483A, G522E, and A626V) in *MYBPC3* were altering the evolutionarily conserved native amino acids, accounted for 8.7% and 6.3% in HCM and DCM, respectively. The bioinformatic tools predicted that those 7 missense mutations were pathogenic. Moreover, the co-segregation of those 7 mutations in families further confirmed their pathogenicity. Remarkably, we also identified compound mutations within the *MYBPC3* gene of 6 cardiomyopathy patients (5%) with more severe disease phenotype; of which, 3 were HCM (2.6%) [(1. K244K + E258K + (IVS6+2T) T>G); (2. L104M + G522E + A626V); (3. P186P + G522E + A626V]; and 3 were DCM (2.4%) [(1. 5'UTR + A392T; 2. V158M+G522E; and 3.V158M + T262T + A626V].

Conclusion: The present comprehensive study on *MYBPC3* has revealed both single and compound mutations in *MYBPC3* and their association with disease in Indian Population with Cardiomyopathies. Our findings may perhaps help in initiating diagnostic strategies and eventually recognizing the targets for therapeutic interventions.

Keywords: *MYBPC3*, cardiomyopathy, sarcomere genes, HCM, DCM

Introduction

Cardiac Myosin Binding Protein C (MyBP-C_OMIM-600958), one of the thick filaments exhibited across the C zone of A-bands of sarcomeres, binds *ß*-myosin (*ß-MYH7_OMIM-160710)* in thick filaments and titin (*TTN_OMIM-188840)* in elastic filaments.[1,2] It serves as a control that limits cross-bridge interactions between myosin and actin.[3,4] Phosphorylation of *MYBPC3* modulates contraction and is believed to play both structural and regulatory functions. A total of 3 isoforms of MyBP-C (a cardiac and two skeletal) have been reported, all 3 share a conserved region composed of 7 IgI (immunoglobulin) and 3 FnIII (fibronectin type III) domains.[4] The cardiac isoform cMyBP-C contains a unique IgI domain (C0) at the N-terminus and four distinctive phosphorylation sites and an exclusive proline-rich 25 residue insertion at the C5 domain.[5,6] Cardiomyopathy (CM), a heart muscle disease, is classified by its morphological features leading to subtypes called hypertrophic (HCM), dilated (*DCM*), left ventricular noncompaction (LVNC), restrictive (RCM), and arrhythmogenic right ventricular cardiomyopathy (*ARVD/C*).[7,8] The former two (HCM and

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DCM) are the most frequent forms of cardiomyopathies, usually affecting the cardiac wall thickness, chamber size and ultimately pumping efficiency.[9] Describing features of HCM include a hypertrophied/thickened left ventricle with weakened diastolic relaxation, myocyte disarray, and replacement fibrosis, with an estimated prevalence of 1 in 500.[10] HCM is known as a "disease of the sarcomere". Sarcomere consists of thick filaments of myosin and thin filaments of actin, tropomyosin, troponin complex, along with the assembly proteins cardiac myosin binding protein C and titin. To date, hundreds of mutations in sarcomere genes have been reported to cause cardiomyopathies,[10–24] most of the mutations (~75%) were found in HCM and a few mutations (~10–16%) were in hereditary DCM. Mutations were predominantly reported in two sarcomere genes: *ß*-Myosin heavy chain *(MYH7)* and Myosin binding protein *C (MYBPC3)*. Defining characteristics of DCM are a left ventricular dilatation, myocardial fibrosis, and myocyte disease, which affect systolic function with an estimated prevalence of 1 in 2500.[13,17] Currently, more than 40 genes, TTN,[25] LMNA,[26] DES[27] RBM20, etc.,[28] along with sarcomere genes were reported to cause familial DCM.[2,14,29–35] The essence of mutation screening in disease genetics mostly relayed on the interpretation of genotype and phenotype correlation. Sometimes the factors that govern the variable phenotypic expressions are largely due to unknown factors like epigenetics, environment, lifestyle, etc., which may also possibly implicate a significant role in disease phenotypes.[9,36] Mutations in myosin binding protein *C (MYBPC3)* gene, one of the most frequent causes of cardiomyopathies, studied extensively in various other populations[9–19,22,36] but not much studied in the Indian population with cardiomyopathies.[37,38] Therefore, here, we performed a targeted screening of the *MYBPC3* gene in 242 cardiomyopathy patients against 197 controls (ethnically matched healthy individuals).

Materials and Methods

Study Population and Ethical Approval

The Institutional Ethical Committees (IECs) of CSIR-Centre for Cellular and Molecular Biology (CCMB) and other two participating hospitals [(1) Government Rajaji Hospital (GH), Madurai, India, and (2) Nizam's Institute of Medical Sciences (NIMS), Hyderabad, India] have approved the study (Table 1). We obtained informed written consent from all the participated individuals before sample collection. We then collected ~5.0 mL of blood samples from each of the 242 patients consisting of 115 HCM, and 127 DCM along with 197 ethnically matched controls (who are healthy individuals without heart problems) (Table 1). To get permission to research human subjects the required guidelines and regulations were followed according to the principles outlined in the Declaration of Helsinki, the World Medical Association.

Genomic DNA

DNA was isolated from the peripheral blood of all cardiomyopathy patients and normal healthy controls using a standard protocol as follows. To the peripheral blood (5.0mL), we added 15mL of erythrocyte lysis buffer [containing 10mM Tris at pH 8.0, 320mM Sucrose, 5mM MgCl2, and 1% Triton X-100; Sigma Chemical Company, St. Louis, MO] for 5 minutes to lysis the erythrocytes. Leucocytes were pelleted by spinning for 5 minutes at 500g in a centrifuge. Leucocytes were lysed using 8mL of leucocyte lysis buffer (400mM Tris, 60mM EDTA, 150mM NaCl, and 1% SDS; Sigma Chemical Company). To this lysate, 2.0mL of 5M sodium perchlorate (E. Merck, Darmstadt, Germany) was added and mixed thoroughly for 2–3 min. DNA was precipitated using isopropanol, after extracting once with phenol:chloroform (1:1) and then with chloroform. DNA was washed 2 times with 70% ethanol, and the pellet was dissolved in the TE (10mM Tris at pH 8.0 and 1mM EDTA) buffer.

Polymerase Chain Reaction (PCR) and Sequence Analysis

The targeted primer sequences for PCR (Supplement Table S1) covering the exons, exon-intron boundaries of *MYBPC3* were designed and synthesized using an ABI 392 oligo synthesizer (Perkin–Elmer, Foster City, CA). Using 50ng of genomic DNA as a template, Polymerase Chain Reactions (PCR) were carried out using 5pM of both forward and reverse primers (Supplementary Table S1), 200mM dNTPs, 10X PCR buffer containing 1.5mM MgCl2, and 1U of AmpliTaq Gold (Perkin–Elmer). Amplifications were carried out in a thermal cycler (MJ Research, Waltham, MA, USA) using the following cycling conditions: 94°C for 5 min, 35 cycles at 94°C for 1 min, 55–60°C for

Table 1 Baseline Clinical Characteristics of HCM and DCM Patients along with Controls

Baseline Characteristics	HCM (N=115)	DCM (N=127)	Controls (N=197)
Age (Yrs)	49±10	48±12	51.0 ±0.2
Sex, males %	67	69	70
Consanguinity %	80.2	35.6	0
Dyspnea or shortness of breath %	65	69.2	0
Angina pectoris (chest pain) %	54	56	0
Syncope (fainting) %	33	30	0
Abnormal ECG %	62	68	0
LVEDD, mm	35± 7.8	67±10	51.5± 2.7
LVESD, mm	20.3 ± 4.7	54 ± 7.7	32.2± 1.2
Septum, mm	22.2 ± 5.3	6 ± 2.7	9.0 ± 0.4
Family History %	82	79	0
Sudden cardiac death %	28.8	14.2	0
LVEF %	49 ±7	31 ± 6.6	64.2 ± 5.1
NYHA Class III &IV	29	35.2	0

Abbreviations: NYHA, New York Heart Association; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; ECG, electrocardiogram; LVEF, left ventricular ejection fraction; SCD, sudden cardiac death.

1 min, 72°C for 1 min, followed by a final extension at 72°C for 10 min. Resulted PCR products (amplicons) were checked using 2% agarose gel electrophoresis. The PCR products were then purified using ExoSAP-IT enzyme (USB Corporation, USA) and subjected to cycle sequencing reaction in GeneAmp 9700 thermal cycler using Big Dye Terminator ready reaction cycle sequencing kit (Applied Biosystems, Foster City, USA). The cycle sequencing products were precipitated with ethanol, dried and dissolved in Hi-Di formamide, and bi-directional sequencing was performed in an ABI 3730XL automated DNA analyzer (Applied Biosystems, Foster City, USA). The *MYBPC3* gene sequences obtained were noted and aligned with the Reference *MYBPC3* gene sequences using Sequence Analyzer and Auto Assembler tools. We followed the American College of Medical Genetics and Genomics (ACMG) guidance for the interpretation of sequence variants.[39] All the mismatched nucleotide sequences were carefully noted and compared against 197 healthy control sequences to detect their significance. We used two bioinformatics tools, Polyphen-2 (Polymorphism phenotyping 2)[40] and SIFT (Sorting intolerant from tolerant),[41] to predict the possible pathogenic effects of missense mutations.

Results

In the present study, we identified 34 genetic variants in *MYBPC3* gene (Table 2), of which 19 were novel (https://www.ncbi.nlm.nih.gov/SNP/snp_viewTablecgi?handle=THANGARAJ_DEEPA_CCMB). We found a splice site mutation [(IVS6+2T) T>G] and 16 were missense mutations [5 in HCM (E258K, T262S, H287L, R408M, V483A), 4 in DCM (T146N, V321L, A392T, E393K) and 7 in both HCM and DCM (L104M, V158M, S236G, R272C, T290A, G522E, A626V)], but all those 16 mutations were absent in 197 controls (Figure 1A and B, Supplementary Figure S1; Table 2). We found 7 out of 16 heterozygous missense mutations in *MYBPC3* [V158M, E258K, R272C, A392T, V483A, G522E, and A626V in *MYBPC3* (Figure 1C and Table 3)] were altering the native evolutionarily conserved amino acids (Figure 1D). The Polyphen-2 or SIFT bioinformatics tools predicted that those 7 missense mutations were pathogenic (Table 2). The co-segregation of those 7 missense mutations in families also confirmed their pathogenicity, accounting for

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Table 2 Comparing the Allele Frequencies of Detected Mutations in the *MYBPC3* Gene of Indian Population vs Other Populations

	Chromosome Position	Position	Major>Minor Allele	Location	SNP Reference	AA Change	PolyPhen-2	SIFT	Predictions	CON/ 197	HCM/ 115	DCM/ 127	IN_AF	ASN_AF	AFR_AF	AMR_AF	EUR_AF	Mean India	Mean ASN-EUR
1	1,147,374,209	g.1045	C>T	5'UTR	Novel-ss6322063401	-	-	-	-	0	0	0.28	0	0	0	0	0	0.001	0
2	1,147,374,129	g.1125	G>T	Intron 1	Reported	-	-	-	-	0	0	0.28	0	0	0	0	0	0.001	0
3	1,147,372,908	g.2346	C>T	Exon 2	Reported	A58A	-	-	-	0	0	0.28	0	0	0	0	0	0.001	0
4	1,147,372,749	g.2505	G>C	Intron 2	rs3729985	-	-	-	Polymorphic	0.73	3.3	10.5	0.03	0.06	0.01	0.03	0.03	0.048	0.045
5	1,147,372,149	g.3105	C>A	Exon 3	Reported	L104M	Benign	Damaging	-	0	0.5	0.28	0	0	0	0	0	0.002	0
6	1,147,371,633	g.3621	C>A	Exon 4	Novel-ss6322063400	T146N	Benign	Tolerated	Benign	0	0	0.28	0	0	0	0	0	0.001	0
7	1,147,371,632	g.3622	C>A	Exon 4	Novel-ss6322063399	T146T	-	-	-	0	0	0.28	0	0	0	0	0	0.001	0
8	1,147,371,620	g.3634	C>A	Exon 4	Novelss6322063398	P150P	-	-	-	0	0	0.28	0	0	0	0	0	0.001	0
9	1,147,371,608	g.3656	G>A	Exon 4	rs3729986	V158M	Possibly Damaging	Damaging	Pathogenic	0	0.75	0.56	0	0	0	0	0	0.003	0
10	1,147,371,442	g.3812	C>T	Exon 5	rs11570051	A179S	Benign	Tolerated	Polymorphic	0	0.25	0	0.02	0.004	0.002	0.07	0.03	0.001	0.01675
11	1,147,371,421	g.3833	G>T	Exon 5	Novel_ss6322063397	P186P	-	-	-	0	0.50	0	0	0	0	0	0	0.001	0
12	1,147,370,041	g.5213	A>G	Exon 6	rs3729989	S236G	Benign	Tolerated	Polymorphic	0	0.25	0.56	0.09	0.02	0.07	0.1	0.14	0.004	0.08
13	1,147,370,029	g.5225	G>A	Exon 6	Reported	E240K	Probably Damaging	Tolerated	-	0	0.25	0	0	0	0	0	0	0.001	0
14	1,147,370,015	g.5239	G>A	Exon 6	Novel_ss6322063396	K244K	-	-	-	0	0.25	0	0	0	0	0	0	0.001	0
15	1,147,369,975	g.5279	G>A	Exon 6	CM981322	E258K	Possibly Damaging	Damaging	Pathogenic	0	0.25	0	0	0	0	0	0	0.001	0
16	1,147,369,973	g.5281	T>G[IVS6+2T]	Intron 6	Novel_ss6322063395	SD	-	-	Pathogenic	0	0.25	0	0	0	0	0	0	0.001	0
17	1,147,369,445	g.5809	A>T	Exon 7	Novel-ss6322063394	T262S	Benign	Tolerated	Benign	0	0.25	0	0	0	0	0	0	0.001	0
18	1,147,369,443	g.5811	C>T	Exon 7	rs11570058	T262T	-	-	Polymorphic	0	1.02	1.4	0.07	0.02	0.03	0.1	0.13	0.008	0.075
19	1,147,369,415	g.5839	A>T	Exon 7	rs397516075	R272C	Probably Damaging	Damaging	Pathogenic	0	0.25	0.28	0	0	0	0	0	0.002	0
20	1,147,369,009	g.6232	A>T	Exon 9	Novel-ss6322063392	H287L	Benign	Damaging	-	0	0.25	0	0	0	0	0	0	0.001	0
21	1,147,369,014	g.6240	A>G	Exon 9	Reported	T290A	Benign	Tolerated	Benign	0	0.25	0.28	0	0	0	0	0	0.002	0
22	1,147,369,022	g.6245	G>A	Exon 9	Novel-ss6322063393	G291G	-	-	-	0	0	0.28	0	0	0	0	0	0.001	0
23	1,147,368,195	g.7059	C>T	Exon 10	rs200713257	D303D	-	-	-	0	0	0.28	0	0	0	0	0	0.001	0
24	1,147,367,887	g.7367	G>C	Exon 11	Novel-ss6322063391	V321L	Benign	Damaging	-	0	0	0.28	0	0	0	0	0	0.003	0
25	1,147,365,092	g.10162	G>A	Exon 12	Novel-ss6322063390	A392T	Probably Damaging	Damaging	Pathogenic	0	0	0.56	0	0	0	0	0	0.003	0
26	1,147,365,089	g.10165	G>A	Exon 12	Novel-ss6322063389	E393K	Benign	Damaging	-	0	0	0.56	0	0	0	0	0	0.003	0

27	1,147,365,060	g.10194	G>A	Exon 12	Novel-ss6322063388	E402E	-	-	-	0	0.51	1.13	0	0	0	0	0	0.005	0
28	1,147,365,043	g.10211	G>T	Exon 12	Novel-ss6322063387	R408IM	Possibly Damaging	Damaging	Pathogenic	0	0.25	0	0	0	0	0	0	0.001	0
29	1,147,365,014	g.10240	G>A	Intron 12	rs11570078	-	-	-	Polymorphic	0	0	0.28	0.09	0.06	0.03	0.1	0.14	0.001	0.1
30	1,147,364,392	g.10862	G>A	Exon 14	Novel-ss6322063386	A482A	-	-	-	0	0.51	0	0	0	0	0	0	0.002	0
31	1,147,364,387	g.10867	T>C	Exon 14	Novel-ss6322063385	V483A	Probably Damaging	Damaging	Pathogenic	0	0.25	0	0	0	0	0	0	0.001	0
32	1,147,364,185	g.11069	G>A	Exon 15	Novel-ss6322063384	G522E	Probably Damaging	Damaging	Pathogenic	0	0.76	0.85	0	0	0	0	0	0.005	0
33	1,147,363,535	g.11719	G>A	Intron 16	Novel-ss2137544465	-	-	-	-	0	0	0.85	0	0	0	0	0	0.003	0
34	1,147,362,650	g.12548	C>T	Exon 20	Reported	A626V	Probably Damaging	Damaging	Pathogenic	0	1.27	0.85	0	0	0	0	0	0.007	0

Note: Reverse strand sequences https://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=THANGARAJ_DEEPA_CCMB.

Abbreviations: SNP, single nucleotide polymorphism; AA, amino acids; rs#.No, reference SNP number; PolyPhen-2, Phenotyping v2; SIFT, sorting intolerant from tolerant; CON, controls; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; IN_AF, Allele frequency of Indian Ancestry; ASN_AF, Allele frequency of Asian Ancestry; AFR_AF, Allele frequency of African Ancestry; AMR_AF, Allele frequency of American Ancestry; EUR_AF, Allele frequency of European Ancestry.

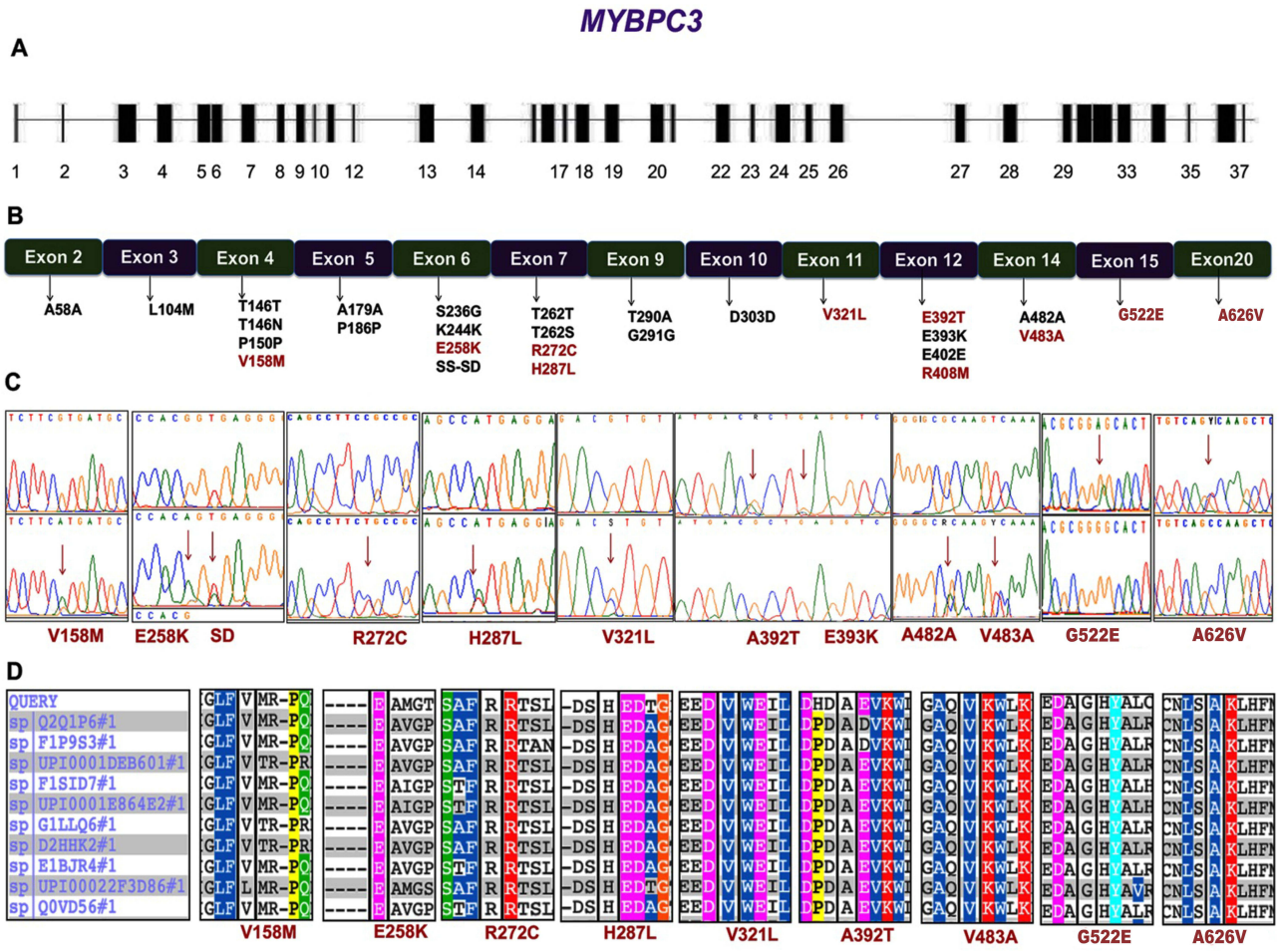

Figure 1 (**A**) Schematic representation of the *MYBPC3* structure. (**B**) Highlighted are the observed exonic, and splice sites variations. (The 10 amino acid substitutions, and 1 splice-site mutation were indicated in red color). (**C**) Electropherograms (arrows) showing 10 missense mutations [V158M, E258K, R272C, H287L, V321L, E392T, R408M, V483A, and a G522E, A626V), and a splice-donor mutation (T>G[IVS6+2T]) in the *MYBPC3* gene. (**D**) Multiple alignments of amino acid sequences in the *MYBPC3*gene of several species, showing that those were highly conserved across many species.

8.7% and 6.3% in HCM and DCM, respectively. Interestingly, in the present study, 6 patients [3 HCM and 3 DCM (Table 4)] with more severe disease phenotypes have shown compound mutations in the *MYBPC3* gene. They were as follows; one was a 51-year-old HCM patient, who carried allelic heterogeneity by possessing three heterozygous mutations in the *MYBPC3*; [a splice site mutation (IVS6+2T) T>G, a missense mutation E258K, and a silent mutation K244K (Figure 1C)]. Another was a 53-year-old HCM patient with three missense mutations; L104M, G522E, and A626V. The third was a 49-year-old HCM patient, who possessed two missense mutations, G522E, A626V, and a silent mutation P186P. The following are the 3 dilated cardiomyopathy patients with more than one mutation in the *MYBPC3* gene. The 1st DCM patient carried a 5'UTR mutation and a missense mutation A392T. The 2nd DCM patient carried 2 missense mutations: V158M and G522E. The 3rd DCM patient possessed 3 missense mutations: V158M, T262T and A626V (Figure 1 and Table 3). In addition, we too detected 10 silent mutations exclusively in cardiomyopathy patients [3 in HCM (P186P, K24K, and A482A), 5 in DCM; A58A, T146T, P150P, G291G, D303D, 2 in both HCM and DCM; T262T and E402E], with unknown implication (Figure S1; Table 2).

Discussion

One of the interesting outcomes of the present study was the identification of compound variations within the *MYBPC3* gene of 6 cardiomyopathy patients with more severe disease presentation. Of which, 3 were HCM [(1. K244K + E258K + (IVS6+2T) T>G), (2. L104M + G522E + A626V) and (3. P186P + G522E + A626V)]; and 3 were DCM [(1. 5'UTR +

Table 3 Missense Mutations in Myosin Binding Protein C (*MYBPC3*) Gene

S:NO	Chromosome Position	Position	Major>Minor Allele	Location	SNP Reference	AA change	PolyPhen-2	SIFT	CON 197	HCM 115	DCM 127
1	1,147,371,608	g.3656	G>A	Exon 4	rs3729986	V158M	Possibly Damaging	Damaging	-	2	1
2	1,147,369,975	g.5279	G>A	Exon 6	CM981322	E258K	Possibly Damaging	Damaging	-	2	-
3	1,147,369,415	g.5839	A>T	Exon 7	rs397516075	R272C	Probably Damaging	Damaging	-	1	1
4	1,147,365,092	g.10162	G>A	Exon 12	Novel-ss6322063390	A392T	Probably Damaging	Damaging	-	-	2
5	1,147,364,387	g.10867	T>C	Exon 14	Novel-ss6322063385	V483A	Probably Damaging	Damaging	-	1	-
6	1,147,364,185	g.11069	G>A	Exon 15	Novel-ss6322063384	G522E	Probably Damaging	Damaging	-	2	2
7	1,147,362,650	g.12548	C>T	Exon 20	rs1352376969	A626V	Probably Damaging	Damaging	-	2	2
Total									-	10/115= 8.7%	8/127= 6.3%

Abbreviations: SNP, single nucleotide polymorphism; AA, amino acids; rs#.No, reference SNP number; PolyPhen-2, Phenotyping v2; SIFT, sorting intolerant from tolerant; CON, controls; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy.

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Table 4 Compound Mutations in Myosin Binding Protein C (*MYBPC3*) Gene

S:No	SNP_AA Change	CON	HCM	DCM
1	K244K, E258K, T>G[IVS6+2T]	–	1(HU22)	–
2	L104M, G522E, A626V	–	1(HU1)	–
3	P186P, G522E, A626V	–	1(HU28)	–
4	5'UTR, A392T, E393K	–	–	1(DU79)
5	V158M, G522E	–	–	1(DU106)
6	V158M+T262T+A626V	–	–	1(DU99)

Abbreviations: SNP, single nucleotide polymorphism; AA, amino acids; CON, controls; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy.

A392T, (2. V158M+G522E), and (3. V158M + T262T + A626V)] (Table 2 and Figure 1), accounting for 2.6% and 2.4% in Indian HCM and DCM patients, respectively. Further analysis of family members of all 6 patients with compound variations has demonstrated the segregation of those variations along with the disease. When we extracted clinical data from hospital records, we noticed increased susceptibility to ventricular arrhythmias in affected patients and a history of sudden cardiac deaths (SCDs) in the families. Studies suggested that the patients possessing compound variations in *MYBPC3*,[21,42,43] have shown dosage-dependent effects,[42,44–46] therefore, required constant monitoring to avoid adverse outcomes.[36,47–49]

We studied a few familial samples to understand the co-segregation of variations in families and their association with disease phenotypes. As, the genotype of the patient's family members are extremely important to understand the genotype-phenotype correlation,[23,33,34] and how it might modify the clinical course and prognosis of the disease along with other factors like epigenetics, lifestyle, environment, etc.[50–53] We understood from our study that most of the patient's family members possessing mutations in *MYBPC3* gene did not show any symptom when they were below 15 years of age, and they all started having the symptom in their 3rd decade of life, ie, late onset of the disease symptoms. Therefore, we too strongly suggest that the mutations in *MYBPC3* showed slightly lower penetrance, delayed onset, and milder forms of disease progression.[48,54,55]

Studies reported deletions, insertions, and splice site variations in the *MYBPC3* gene.[56–61] In our previous study on *MYBPC3*, we observed a founder 25bp del in *MYBPC3* of HCM, DCM, and RCM and evaluated its distribution among the South-Asian population, and pinpointed an association with familial cardiomyopathies with an increased chance of heart failure [overall OR, 6.99; $p = 4 \times 10 (-11)$].[23] In the present study, we report 16 missense mutations in the *MYBPC3* gene of Indian cardiomyopathy patients and those were absent in 197 normal healthy controls (Table 2 and Figure 1A). Remarkably, we found that 7 out of 16 heterozygous missense mutations in *MYBPC3* (V158M, E258K, R272C, A392T, V483A, G522E, and A626V) (Figure 1C) were altering the native evolutionarily conserved amino acids (Figure 1D), accounting for 8.7% and 6.3% in HCM and DCM, respectively. Polyphen-2 or SIFT bioinformatics tools also predicted that those 7 missense mutations were pathogenic (Table 2). The co-segregation of those mutations in families also confirmed their pathogenicity. Except for two missense mutations, R272C and V483A, the remaining 5 missense mutations (V158M, E258K, A392T, G522E, and A626V) were also detected along with other variations in the *MYBPC3* gene as compound mutations (Table 4).

In our previous studies, we reported a few genetic variations in other sarcomere genes of Indian cardiomyopathy patients: Tropomyosin (α-*TMP1)*,[45] Troponin I3 (*TNNI3*),[24,62] Troponin T2 (*TNNT2*),[34,63] Actin (*ACTC*),[64] Myosin (*β-MYH7)*,[35,65,66] and *MYL2 & MYL3*.[67] Therefore, our present and previous studies clearly illustrated the prevalence and spectrum of variations in sarcomere genes and their associations in Indian populationwith cardiomyopathies.[23,24,34,35,45,62–67]

Our categorization as pathogenic variations relied on the result that we could detect those missense mutations exclusively in patients and their family members. In the present study, we found 19 novel SNPs in the *MYBPC3* gene, along with accumulated compound variations that were responsible for more severe disease phenotypes in Indian

cardiomyopathies. Our present comprehensive genetic analysis of the *MYBPC3* gene in Indian HCM and DCM patients has given important insight into risk stratification. Based on our present and previous studies on sarcomere genes in Indian population,[23,24,34,35,45,62–67] we fully agree that the mutations in myosin heavy chain and myosin binding protein C are the frequent causes of cardiomyopathies; therefore, these two genes should be screened first, secondly, the thin filament regulatory genes (*TNNT2, TNNI3*, and *TPM1*), and finally, the rarely involved genes like *TTN, MYL2, MYL3*, and *ACTC*. Importantly, it is not sufficient and advisable to screen only the known reported mutations, because we could miss unique, rare, and population-specific disease-causing mutations.

Limitations of the Study

For any genetic study, it is crucial to extend the study with their family members to understand the inheritance pattern and to correlate the mutations with the disease phenotype. However, generally, it is tough to collect the samples from family member for genetic analysis, mainly because the patients are not strictly adhering to the follow-up procedures that would have allowed us to invite the family members for counselling and further genetic studies. Though we identified missense mutations in many individuals, we could not establish the genotype-to-phenotype correlation within the family in many cases. Thus, for some patients, performing genetic testing, understanding their family history, and giving counselling is not an easy task!

Conclusion

The present comprehensive study has revealed both single and compound mutations in *MYBPC3* and their association with disease in Indian population with cardiomyopathies. Our findings may perhaps help in initiating diagnostic strategies and eventually recognizing the targets for therapeutic interventions.

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Disclosure

The authors report no conflicts of interest in this work.

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