Original Article

Novel Mutations in β-MYH7 Gene in Indian Patients With Dilated Cardiomyopathy

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ABSTRACT

Background: Heart failure is a hallmark of severe hypertrophic cardiomyopathy and dilated cardiomyopathy (DCM). Several mutations in the β-MYH7 gene lead to hypertrophic cardiomyopathy. Recently, causative mutations in the β-MYH7 gene have also been detected in DCM from different populations.

Methods: Here, we sequenced the β-MYH7 gene in 137 Indian DCM patients and 167 ethnically matched healthy controls to detect the frequency of mutations and their association.

RESULTS

Morbidity and mortality rates associated with cardiovascular disease are very high in South Asia. India is a country known for its large ethnic diversity and serves as a potential source for understanding population-specific genetic variations.1 Cardiomyopathy affects the myocardium of the heart and increases the risk of heart failure and sudden cardiac death.2

Hypertrophic cardiomyopathy and dilated cardiomyopathy (DCM) are the 2 common types of cardiomyopathy classified according to heart morphology.2 The characteristic features of DCM include left ventricular dilatation, systolic dysfunction, arrhythmias (irregular heartbeats), dyspnea (shortness of breath), and heart murmurs, with an estimated prevalence of 1:2500.3,4 Studies have reported an association of DCM with dozens of mutations in more than 40 genes.5 A large proportion of those mutations predominantly encoded for sarcomere and cytoskeletal protein.6-10

The thick cardiac filament β-myosin heavy chain (β-MYH7) is one of the major sarcomere genes (22,883 base pairs) mapped on the long arm (q12) of chromosome 14 and encoded by 40 exons.11 The first 2 exons are noncoding; however, the 37th and 38th exons have been fused without intron.12 Interestingly, the causative mutation for hypertrophic cardiomyopathy was first detected in the β-MYH7 gene.13 The β-myosin heavy chain (β-MYH7) and myosin binding protein C (MYBPC3)14,15,17-19 are the 2 most commonly affected sarcomere genes, of which ~50% have

RÉSUMÉ

Introduction : L’insuffisance cardiaque est une caractéristique de la cardiomyopathie hypertrophique grave et de la cardiomyopathie dilatée (CMD). Plusieurs mutations dans le gène β-MYH7 conduisent à la cardiomyopathie hypertrophique. Récemment, les mutations causales dans le gène β-MYH7 ont également été détectées au sein de différentes populations atteintes de CMD.

Méthodes : Ici, nous avons séquencé le gène β-MYH7 de 137 patients indiens atteints de CMD et de 167 témoins sains appariés...
Results: Our study revealed 27 variations, of which 7 mutations (8.0%) were detected exclusively in Indian DCM patients for the first time. These included 4 missense mutations—Arg723His, Phe510Leu, His358Leu, and Ser384Tyr (2.9%); a frameshift mutation—Asn676del (1.5%); and 2 splice-site mutations (IVS17-2T) T→G and (IVS19-1G) G→A (3.6%). Remarkably, all 4 missense mutations altered evolutionarily conserved amino acids. All 4 missense mutations were predicted to be pathogenic by 2 bioinformatics tools—polymorphism phenotyping v2 (PolyPhen-2) and sorting intolerant from tolerant (SIFT). In addition, the 4 homology models of phenotyping v2 (PolyPhen-2) and sorting intolerant from tolerant (SIFT). All 4 missense mutations were violations of w.Conclusions: ciency.13,28 The clinical heterogeneity, ranging from asymp-
del (1.5%); and 2 splice-site mutations (IVS17-2T) T→G and (IVS19-1G) G→A (3.6%). Remarkably, all 4 missense mutations altered evolutionarily conserved amino acids. All 4 missense mutations were predicted to be pathogenic by 2 bioinformatics tools—polymorphism phenotyping v2 (PolyPhen-2) and sorting intolerant from tolerant (SIFT). All 4 missense mutations were violations of w.

Results: Our study revealed 27 variations, of which 7 mutations (8.0%) were detected exclusively in Indian DCM patients for the first time. These included 4 missense mutations—Arg723His, Phe510Leu, His358Leu, and Ser384Tyr (2.9%); a frameshift mutation—Asn676del (1.5%); and 2 splice-site mutations (IVS17-2T) T→G and (IVS19-1G) G→A (3.6%). Remarkably, all 4 missense mutations altered evolutionarily conserved amino acids. All 4 missense mutations were predicted to be pathogenic by 2 bioinformatics tools—polymorphism phenotyping v2 (PolyPhen-2) and sorting intolerant from tolerant (SIFT). In addition, the 4 homology models of β-MYH7—p.Leu358, p.Tyr384, p.Leu510, and p.His723—displayed root-mean-square deviations of ~2.55 Å, ~1.24 Å, ~3.36 Å, and ~3.86 Å, respectively.

Conclusions: In the present study, we detected numerous novel, unique, and rare mutations in the β-MYH7 gene exclusively in Indian DCM patients (8.0%). Here, we demonstrated how each mutant (missense) uniquely disrupts a critical network of non-bonding interactions at the mutation site (molecular level) and may contribute to development of dilated cardiomyopathy (DCM). Therefore, our findings may provide insight into the understanding of the molecular bases of disease and into diagnosis along with promoting novel therapeutic strategies (through personalized medicine).

Materials and Methods

Ethical statement and samples

We enrolled 137 patients with DCM from (i) CARE Hospitals, Hyderabad and (ii) Government Rajaji Hospital, Madurai, Tamil Nadu, for our study, after they underwent complete medical and physical examination (Table 1). The Institutional Ethical Committees (IEC); the Council of Scientific and Industrial Research-Centre for Cellular and Molecular Biology (CCMB), Hyderabad; the CARE Hospitals, Hyderabad, Telangana; and the Government Rajaji Hospital, Madurai, Tamil Nadu, approved the study. We also recruited a total of 167 healthy volunteers, matched to patients for age, sex, and ethnicity as controls, provided that they had a normal electrocardiogram and echocardiography measurements and were unrelated to the patients with DCM (Table 1). The blood samples of both patients and controls were collected with informed written consent.

We followed the relevant guidelines36,37 to diagnose and manage DCM as given on the website www.genetests.org.38 We recommend clinical screening of first-degree relatives (children, siblings, and parents) at risk of developing DCM (eg, as determined by echocardiogram, electrocardiogram,
historical, exam, etc.). We have provided counselling to patients with a heritable genetic basis and discussed its likely inheritance pattern, the typical age of onset, presenting symptoms, and other relevant features according to the available guidelines for DCM.\textsuperscript{36,37} We carried out all our investigations according to the guidelines and regulations regarding research on human subjects, and we have followed the ethics requirements of the Declaration of Helsinki and the World Medical Association.

### Genetic studies

Peripheral blood samples from patients and controls were collected, and DNA was extracted, sequenced, and screened for variations by methods described elsewhere.\textsuperscript{39,40} Primer sequences covering all the exons and their flanking areas in the β-MYH7 gene were synthesized. We then carried out polymerase chain reaction (PCR) analysis under standard conditions, using 50 ng of genomic DNA template, 5 pmol each of forward and reverse primers, 1U of Taq DNA polymerase, 200 μM of deoxynucleoside triphosphates, 1X PCR buffer containing 1.5 mM MgCl₂, and water for a total of 20 μL. The desired genomic fragments were amplified using the PCR machine (MJ Research Thermal cycler, Waltham, MA), with the initial denaturation at 94°C for 4 minutes, followed by cycling conditions: 35 cycles at 94°C for 50 seconds, 55-60°C for 45 seconds, 72°C for 55 seconds, and a final extension at 72°C for 9 minutes. The PCR amplicons were purified using Exonuclease 1 and Shrimp alkaline phosphatase, following the manufacturer instructions (USB Corporation, Cleveland, OH). The purified amplicons were sequenced bi-directionally using the ABI Big Dye terminator cycle sequencing kit (Perkin-Elmer, Foster City, CA) and the ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). The amplicon sequences were assembled and screened for variations against the respective reference sequence (Gen-Bank) using Auto-Assembler software (Applied Biosystems, Foster City, CA).

### In silico analyses

The possible pathogenic effects of missense mutations were predicted using 2 bioinformatics tools—PolyPhen-2 \textsuperscript{41} and SIFT.\textsuperscript{42} In addition, the homology models were built for all novel missense mutations by SWISS-MODEL homology-modelling (swissmodel.expasy.org).\textsuperscript{43} We used a native 3D template structure with 99% similarity (Supplemental Fig. S1) obtained from the protein data bank (PDB) (www.rcsb.org/pdb/explore/explore.do?structureId=4P7H)\textsuperscript{44} for building homology modelling. To clearly understand the impact of 4 non-synonymous single-nucleotide polymorphisms (nsSNPs) on β-MYH7 protein structure, we first superimposed each of the 4 homology models of β-MYH7 with native β-MYH7 protein template structure to measure the root-mean-square deviations (RMSDs) between the atoms (backbone atoms) of the superimposed pairs. We then studied the non-bonding interactions (created/destroyed) at the mutation site, the distances and interactions contact plots, and Ramachandran plots for each homology model vs native β-MYH7.

### Results

Direct sequence analysis of β-MYH7 in 137 dilated cardiomyopathy (DCM) patients vs 167 healthy controls (Table 1) revealed 27 variations. Of these, 13 were novel (4 nsSNPs, a frameshift, 2 splice sites, and 3 synonymous and 3 intronic variations; Table 2; www.ncbi.nlm.nih.gov/projec/SNP/snp_viewBatch.cgi?sbid=1062022). The β-MYH7 gene encompasses 40 exons (Fig. 1A), in which we detected the following: 4 missense mutations—Arg723His, Phe510-Leu, His358Leu, and Ser384Tyr (2.9%); a frameshift mutation—Asn676_T-del (1.5%); and 13 silent mutations (synSNPs; Fig. 1B). Further, we detected 2 splice-site mutations: one was a splice donor (IVS17+2T) T>G in a DCM patient, and another was a splice acceptor (IVS19-1G) G>A in 4 individuals with DCM, accounting for 3.6% (Fig. 1B). On the whole, a total of 7 mutations (8.0%), consisting of 4 missense mutations, a frameshift, and 2 splice-site mutations (Fig. 1C) were detected for the first time in DCM patients; these were absent in 167 controls. We found that all 4 missense mutations had altered the evolutionarily conserved amino acids (Fig. 1D). Interestingly, 2 DCM patients with missense mutations (a 48-year-old with Arg723His and a 49-year-old with His358Leu) showed allelic heterogeneity by carrying additional mutations in the same locus [Arg723His, (IVS19-1G) G>A and Ala729Ala in exon 20 (Fig. 2A), and His358Leu and a silent mutation, Gly354Gly, in exon 12 (Fig. 2E) of the β-MYH7 gene; Fig. 2].

We observed a 55-year-old DCM patient (consanguineous marriage) and her 32-year-old son with DCM, who were both found to carry a Phe510-Leu missense mutation in the β-MYH7 gene (Fig. 2B), which was absent in healthy controls (Table 2). We detected a splice acceptor mutation [(IVS19-1G) G>A] alone in a 45-year-old DCM patient and his 39-year-old asymptomatic sister with DCM, with a family history of sudden cardiac death (Fig. 2F). Further, we noticed

### Table 1. Baseline clinical characteristics of patients with DCM, along with controls

| Baseline characteristics | DCM patients (n = 137) | Controls (n = 167) |
|--------------------------|------------------------|--------------------|
| Age, y                   | 48 ± 12                | 51.0 ± 0.2         |
| Sex, male                | 69                      | 70                 |
| Consanguinity            | 35.6                    | 0                  |
| Diet: non-vegetarian     | 88.6                    | 67                 |
| Diabetes                 | 25                      | 0                  |
| Hypertension             | 27.2                    | 10                 |
| Dyspnea or shortness of breath | 69.2                | 0                  |
| Angina pectoris (chest pain) | 56                    | 0                  |
| Syncope (fainting)       | 30                      | 0                  |
| Abnormal ECG             | 68                      | 0                  |
| LVESD, mm                | 67 ± 10                 | 51.5 ± 2.7         |
| LVESD, mm                | 54 ± 7.7                | 32.2 ± 1.2         |
| Septum, mm               | 6 ± 2.7                 | 9.0 ± 0.4          |
| Family history           | 79                      | 0                  |
| Sudden cardiac death     | 14.2                    | 0                  |
| LVEF, %                  | 31 ± 6.6                | 64.2 ± 5.1         |
| NYHA Class III & IV      | 35.2                    | 0                  |

Values are % or mean ± standard deviation, unless otherwise indicated.

DCM, dilated cardiomyopathy; ECG, electrocardiogram; LV, left ventricular; LVESD, left ventricular end-systolic dimension; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.
| S.No | Chromosome positions | SNP_SS# No | Major>minor alleles | Locations | SNP_rs# No | Amino acids, net charges | Poly-Phen2 | SIFT | Predictions | CON | DCM | Novel/reported |
|------|----------------------|------------|---------------------|-----------|------------|--------------------------|------------|------|-------------|-----|-----|----------------|
| 1    | 1423901723           | 1505811302 | C>G                 | Intron 5  | rs606231313| —                        | —          | —    | —           | 0   | 1   | Novel          |
| 2    | 1423901719           | 1505811269 | C>T                 | Intron 5  | rs376022200| —                        | —          | —    | —           | 0   | 1   | Reported        |
| 3    | 1423900939           | 1505811271 | G>T                 | Intron 7  | rs369187721| —                        | —          | —    | —           | 0   | 1   | Reported        |
| 4    | 1423900794           | 974488077  | C>T                 | Exon 8   | rs2069542  | F244F                   | 0          | 3    | 7           | 0   | 1   | Reported        |
| 5    | 1423899060           | 974488074  | C>T                 | Exon 12  | rs735712   | G554G                   | 0          | —    | —           | 0   | 1   | Reported        |
| 6    | 1423899049           | 1505811307 | A>T                 | Exon 12  | rs606231316| H358L (~1)              | Damaging   | —    | —           | 0   | 1   | Novel          |
| 7    | 1423898994           | 974488072  | C>T                 | Exon 12  | rs2231126  | D376D                   | —          | —    | —           | 0   | 2   | Reported        |
| 8    | 1423898544           | 1505811310 | C>A                 | Exon 13  | rs606231319| S384Y (0)               | Damaging   | —    | —           | 0   | 1   | Novel          |
| 9    | 1423897757           | 1505811314 | C>A                 | Exon 15  | rs606231323| F310L (0)               | Damaging   | —    | —           | 0   | 1   | Novel          |
| 10   | 1423896543           | 1505811318 | T>A                 | Intron 16| rs606231327| —                        | —          | —    | —           | 0   | 2   | Novel          |
| 11   | 1423896647           | 1505811320 | T>G (IVS17+2T)     | Intron 17| rs606231329| SD                      | —          | —    | —           | 0   | 1   | Novel          |
| 12   | 1423896637           | 1505811287 | G>A                 | Intron 17| rs48325953 | —                        | —          | —    | —           | 0   | 2   | Reported        |
| 13   | 1423896335           | 1505811322 | G>A                 | Intron 17| rs606231330| —                        | —          | —    | —           | 0   | 0   | Novel          |
| 14   | 1423896002           | 1505811323 | T del               | Exon 18  | rs606231331| N676 (fs)               | Damaging   | —    | —           | 0   | 2   | Novel          |
| 15   | 1423896002           | 34238304   | T>C                 | Exon 18  | rs145564868| N676N                   | —          | —    | —           | 0   | 4   | Reported        |
| 16   | 1423895289           | 4041783    | G>C                 | Exon 19  | rs1126621  | G836G                   | 0          | 4    | 9           | 9   | 5   | Reported        |
| 17   | 1423895028           | 1505811326 | G>A (IVS19-1G)     | Intron 19| rs606231334| SA                      | —          | —    | —           | 0   | 4   | Novel          |
| 18   | 1423895022           | 1505811293 | G>A                 | Exon 20  | rs397516135| R723H (+2)              | Damaging   | —    | —           | 0   | 1   | Novel          |
| 19   | 1423895009           | 1505811329 | A>G                 | Exon 20  | rs606231336| P727P                   | —          | —    | —           | 0   | 2   | Novel          |
| 20   | 1423895003           | Reported   | C>A                 | Exon 20  | CM057344   | A729A                   | —          | —    | —           | 0   | 2   | Reported        |
| 21   | 1423894188           | 1505811331 | G>T                 | Exon 22  | rs606231338| G823G                   | 0          | 4    | 9           | 0   | 4   | Reported        |
| 22   | 1423894161           | 1505811332 | C>T                 | Exon 22  | rs606231339| L832L                   | 0          | 4    | 9           | 0   | 4   | Reported        |
| 23   | 1423894132           | 1505811299 | G>A                 | Exon 22  | rs397516154| E875E                   | —          | —    | —           | 0   | 3   | Reported        |
| 24   | 1423893287           | 4041786    | C>T                 | Exon 23  | rs1041957  | A917A                   | 0          | 5    | 7           | 0   | 4   | Reported        |
| 25   | 1423892888           | 974488065  | T>C                 | Exon 24  | rs7157716  | I989I                   | 0          | 4    | 9           | 0   | 4   | Reported        |
| 26   | 1423892819           | 990927921  | C>T                 | Exon 24  | rs145579951| A1012A                  | 0          | 0    | 2           | 0   | 4   | Reported        |
| 27   | 1423891240           | 1505811301 | C>G                 | Intron 25| rs48352965 | —                      | 0          | 2    | —           | 0   | 2   | Reported        |

Missense mutations: 4 of 137 = 2.9%; splice-site mutations: 5 of 137 = 3.6%; frameshift mutations: 2 of 137 = 1.5%; total mutations in DCM patients = 8.0%.

S.No, serial number; CON, controls; PolyPhen-2, pheno-typing v2; rs#.No, reference SNP number; SIFT, sorting intolerant from tolerant; SNP, single nucleotide polymorphism; SS#.No, submitted SNP number.
a splice donor variation [(IVS17 +2T) T>G] in intron 17 of the β-MYH7 in a 33-year-old DCM patient and later in his 27-year-old sister with the disease, along with a family history of sudden cardiac death (Fig. 2C). The splice site variations [T>G (IVS17 +2T) G>A (IVS19-1G)] and a frameshift mutation [N676K (fs)], in the β-MYH7 gene. (D) Multiple alignments of amino acid sequences in the β-MYH7 gene of several species, showing that these 4 amino acids (missense mutations) are highly conserved across many species. A: Query (Homo sapiens; human); B: G1T0I0—Oryctolagus cuniculus (Rabbit); C: F7XKMO—Callithrix jacchus (common marmoset); D: P13533—Homo sapiens (human); E: UP10022877DF—Cavia porcellus (guinea pig); F: G3V885—Rattus norvegicus (rat); G: P02563—Rattus norvegicus (rat); H: P13539—Mesocricetus auratus (golden hamster); I: P02566—Mus musculus (mouse); J: Q27AW4—Mus musculus (mouse); K: FIN2GO—Bos taurus (bovine); L: UP1000157615—Bos taurus (bovine); M: F7GOR4—Macaca mulatta (rhesus macaque); N: UPI0002257BDF—Canis lupus familiaris (dog); and O: G3RLR1—Gorilla gorilla gorilla (Western lowland gorilla).

Figure 1. (A) Schematic representation of the β-MYH7 structure. (B) Highlighted are the observed exonic, splice sites, and frameshift (fs) variations. (The 4 amino acid substitutions, 1 frameshift mutation, and 2 splice-site mutations are indicated in red). (C) Electropherograms (arrows) showing 4 missense mutations [CAC—CTC (p.His358Leu), TCT—TAT (p.Phe510Leu), and CGC—CAC (p.Arg723His)]; 2 splice-site variations [T>G (IVS17 +2T) G>A (IVS19-1G)]; and a frameshift mutation [N676K (fs)], in the β-MYH7 gene. (D) Multiple alignments of amino acid sequences in the β-MYH7 gene of several species, showing that these 4 amino acids (missense mutations) are highly conserved across many species. A: Query (Homo sapiens; human); B: G1T0I0—Oryctolagus cuniculus (Rabbit); C: F7XKMO—Callithrix jacchus (common marmoset); D: P13533—Homo sapiens (human); E: UP10022877DF—Cavia porcellus (guinea pig); F: G3V885—Rattus norvegicus (rat); G: P02563—Rattus norvegicus (rat); H: P13539—Mesocricetus auratus (golden hamster); I: P02566—Mus musculus (mouse); J: Q27AW4—Mus musculus (mouse); K: FIN2GO—Bos taurus (bovine); L: UP1000157615—Bos taurus (bovine); M: F7GOR4—Macaca mulatta (rhesus macaque); N: UPI0002257BDF—Canis lupus familiaris (dog); and O: G3RLR1—Gorilla gorilla gorilla (Western lowland gorilla).
Figure 2. Shown are the pedigrees of dilated cardiomyopathy patients and their family members with novel mutations in the \(\beta\)-MYH7 gene. G, generation; SCD, sudden cardiac death.

Figure 3. The root mean square deviations (RMSDs) of superimposed 3D structure of each of the 4 mutant homology models with native template. L, leucine; H, histidine; S, serine; Y, tyrosine; F, phenylalanine; R, arginine.
We further studied the distance and interaction contact plots with atoms less than 6-12 Å. In the distance contact plots, we investigated the spatial distances between (i) Cα-Cα (Supplemental Table S1; Supplemental Fig. S2), (ii) Cβ-Cβ (Supplemental Table S1; Supplemental Fig. S3), and (iii) side-chain amino acids residues in each homology model vs native template (Supplemental Table S1; Supplemental Fig. S4) to understand the possible atom pairs destroyed/created due to differences in their distances. In interaction contact plots, we studied the possible interactions destroyed/created in each of the 4 (mutant) homology models vs native by comparing their (i) hydrogen bonding (Supplemental Table S1; Supplemental Fig. S5) and (ii) residue-type interactions (Supplemental Table S1; Supplemental Fig. S6). We then studied the Ramachandran plot (Supplemental Table S2; Supplemental Fig. S7) to understand how those altered molecular interactions have destabilized the mutant protein structure. In the Ramachandran plot, we compared the energetically allowed and disallowed regions of backbone dihedral angles ψ against φ of amino acid residues in the homology model vs native (Supplemental Table S2; Supplemental Fig. S7).

**Discussion**

In the present study, we detected 4 novel nsSNPs altering the evolutionarily conserved amino acids in the β-MYH7 gene (Fig. 1D). Further, all 4 nsSNPs of the β-MYH7 gene were predicted to be pathogenic by the PolyPhen-2 41 and SIFT 42 bioinformatics tools (Table 2). In addition, we found that the 4 mutants (homology models) uniquely disrupt and deviate from a critical network of non-bonding interactions at the mutation site (molecular level) and disturb the structure (Table 3; Fig. 4). We know that a network of various kinds of non-covalent interactions between the amino acid residues drives the accurate 3D structure of the protein.

Moreover, the primary amino acid sequences carry all the genetic information that denotes the folding of a protein. In general, when the protein folds most of the nonpolar residues, some of the peptide groups, and a few of the polar and charged side chains are also buried in the interior of the molecule, more specifically out of contact with water, and thus collectively play a crucial role in protein 3D conformation. 45 Although different sequences potentially generate a similar structure, a nsSNP can change protein structure.
| Amino acid REF | Non-bond Distance | Distance labelled | Types | Angle XDA | Angle DAY | Amino acid MUT | Non-bond Distance | Labelled Distance | Types | Angle XDA | Angle DAY |
|----------------|------------------|------------------|-------|-----------|-----------|----------------|------------------|------------------|-------|-----------|-----------|
| HIS358: A:HIS358:N - A:GLY354:O | 3.33 | Conventional hydrogen bond | 108 | 150 | LEU358: A:LEU358:N - A:GLY354:O | 3.34 | Conventional hydrogen bond | 108 | 150 |
| A:HIS358:ND1 - A:GLU380:OE1 | 2.72 | A | Conventional hydrogen bond | 123 | 145 | Bond-destroyed | --- | --- | --- | --- |
| A:HIS358:CD2 - A:GLY354:O | 3.11 | B | Conventional hydrogen bond | 105 | 127 | Bond-destroyed | --- | --- | --- | --- |
| A:HIS358 - A:ILE303 | 5.15 | C | Pi—Alkyl | --- | --- | Bond-destroyed | --- | --- | --- | --- |
| A:HIS358:CE1 - A:LEU302:O | 3.05 | D | Carbon hydrogen bond | 91.4 | 130 | Bond-destroyed | --- | --- | --- | --- |
| A:LYS358 - A:ALG380:OE2 | 4.16 | E1 | Pi—Alkyl | --- | --- | --- | --- | --- | --- | --- |
| A:LYS358: NZ - A:ALG380:OE1 | 3.71 | F1 | Salt bridge | 109 | 121 | A:LEU298 - A:ILE303 | G | 5.46 | Alkyl | --- | --- |
| SER384: A:SER384:OG - A:ALA355:O | 3.35 | A | Conventional hydrogen bond | 104 | 121 | TYR384 | Bond-destroyed | --- | --- | --- | --- |
| A:TYR609:OH - A:MET388:SD | 3.59 | B | Conventional hydrogen bond | 94.7 | 91.3 | Bond-destroyed | --- | --- | --- | --- |
| A:MET388:SD - A:TYR609 | 4.73 | C | Pi—Sulfur | --- | --- | Bond-destroyed | --- | --- | --- | --- |
| A:ALA355 - A:MET388 | 3.92 | Alkyl | --- | --- | --- | --- | --- | --- | --- | --- |
| A:MET388 - A:LEU390 | 5.09 | D | Alkyl | --- | --- | --- | --- | --- | --- | --- |
| NO-BOND | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| NO-BOND | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| NO-BOND | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| NO-BOND | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| PHE510: A:PHE513 - A:LYS707 | 3.34 | B | Pi—Alkyl | --- | --- | --- | --- | --- | --- | --- |
| A:ILE704:N - A:GLU700:O | 3.11 | --- | Conventional hydrogen bond | 113 | 157 | --- | --- | --- | --- | --- |
| A:MET493 - A:ILE704 | 5.46 | C | Alkyl | --- | --- | --- | --- | --- | --- | --- |
| A:MET510 - A:LYS707 | 4.45 | D1 | Pi—Alkyl | --- | --- | --- | --- | --- | --- | --- |
| A:LYS707:NZ - A:ASP516:OD2 | 2.7 | --- | Salt bridge | 119 | 128 | --- | --- | --- | --- | --- |
| A:PHES513 - A:ILE704 | 4.02 | --- | Pi—Alkyl | --- | --- | --- | --- | --- | --- | --- |
| NO-BOND | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ARG723: A:ILE729 - A:LYS723 | 4.92 | C | Alkyl | 158 | 124 | Bond-destroyed | --- | --- | --- | --- |
| A:ARG723:HH12 - A:GLU671:OE2 | 3.36 | D | Salt bridge; attractive charge | 93.3 | 125 | Bond-destroyed | --- | --- | --- | --- |
| A:LYS671:HZ1 - A:GLU671:OE1 | 2.43 | E | Salt bridge; attractive charge | 132 | 115 | Bond-destroyed | --- | --- | --- | --- |
| A:ARG723:HH12 - A:GLU981:O | 3.26 | F | Conventional hydrogen bond | 93.3 | 125 | --- | --- | --- | --- | --- |
| A:AAL729 - A:ARG723 | 4.7 | G1 | Alkyl | --- | --- | --- | --- | --- | --- | --- |
| A:ARG723:HN - A:ARG719:O | 2.1 | --- | Conventional hydrogen bond | 144 | 146 | --- | --- | --- | --- | --- |
| A:ASN726:HN - A:ARG723:O | 2.08 | --- | Conventional hydrogen bond | 145 | 127 | --- | --- | --- | --- | --- |

REF, reference; MUT, mutant; XDA, angular distance at X axis; DAY, angular distance at Y axis.
dramatically and lead to disease pathogenicity. For example, a missense mutation of glutamic acid to valine (E6V) in β-globin was reported to cause sickle cell anemia. Here, in the p.Leu510 homology model of β-MYH7, we found that the mutant Leu510 changes the conformation of a residue Lys766 (one of the crucial residues) at the actin (ligand) binding region of the myosin head domain and may affect actin-binding and cardiac muscle contractile function (Fig. 4F). The cardiac muscle contraction at the molecular level is determined by the time- and space-regulated interactions of myosin head (β-MYH7) with actin (ACTC) at the expense of cyclic adenosine triphosphate hydrolysis, regulated with change in the intracellular free calcium concentration. Thus, the mutant homology model p.Leu510 of β-MYH7 showed evident RMSDs of ~3.36 Å (Fig. 3). Interestingly, the 3 missense mutations Arg723His, Phe510Leu, and His358Leu have been found to co-segregate along with the disease in their families, hence showing a strong association (Fig. 2). Unfortunately, we have not screened family members of a DCM patient with the Ser384Tyr missense mutation, as the samples were not available.

In the p.His723 homology model of β-MYH7, the mutant His723 creates a hydrophobic interaction with Pro727, changing the structure by destroying a hydrophobic interaction between 2 isoleucines—Ile730 and Ile736—2 electrostatic salt bridges, and a conventional hydrogen bond (Fig. 4, G and H; Table 3). We know that the proline residue is unique, that it lacks an amide proton, and that it cannot donate hydrogen to stabilize other bonds or promote stability. As a result, the mutant p.His723 homology model of β-MYH7 showed evident RMSDs of ~3.86 Å (Fig. 3). In the p.Leu358 homology model of β-MYH7, the mutant Leu358 destroys a hydrophobic interaction between His358 and Ile303, but the mutant forms a compensatory hydrophobic interaction between Ile303 and Leu298 (Fig. 4B), within the hydrophobic core that stabilizes the protein structure with moderate RMSDs of ~2.55 Å. In addition, 2 DCM patients (a 48-year-old with Arg723His and a 49-year-old with His358Leu) have shown allelic heterogeneity by carrying additional variations in the same locus (Fig. 2). However, the Ser384Tyr was predicted to be pathogenic by PolyPhen-2 and SIFT, and although it affected the evolutionarily conserved amino acid, the homology model p.Tyr384 of β-MYH7 could not show much deviation in the overall structure, with a least RMSD of ~1.24 Å (Fig. 3). Therefore, the pathogenicity of the mutant needs to be studied further using functional analysis. On the whole, we could not get much information regarding the patient who died from sudden cardiac death or pedigree data of patients for more than 2 generations.

We then plotted distance and interaction contact plots and showed how a single amino acid change could fine-tune the whole protein structure (Supplemental Table S1). The interactions between the constituent amino acids determine the formation of protein structure and its biological activity. The well-organized 3D native conformation of a protein is determined based on the molecular interactions of amino acids in its polypeptide chain; however, incorrect interactions may destabilize a protein structure and implicate diseases. A Ramachandran plot showed the energetically allowed and disallowed regions of backbone dihedral angles ψ against φ of amino acid residues in the homology model vs native (Supplemental Table S2). Some studies suggest that the abnormal protein itself serves as a pathogenic agent and is associated with various diseases. Principally, the 3D structure of the protein regulates its biological activity; any change in the amino acid sequences may affect its folding and cause the accumulation of nonfunctional forms of protein in the formation of oligomers and amyloid fibrils and thus cause diseases such as spongiform encephalopathy, type 2 diabetes, sickle cell anemia, Alzheimer disease, Creutzfeldt—Jakob disease, Parkinson disease, and prion disease. Overall, we detected a total of 7 novel causative mutations (8.0%), consisting of 4 missense mutations, a frameshift, and 2 splice-site mutations (Fig. 1C) for the first time in Indian DCM patients; these were all absent in 167 controls. We previously reported a few variations in myosin-binding protein C (MyBPC3), troponin 13 (TNNI3), troponin T2 (TNNT2), actin (ACTC), and myosin (β-MYH7) in Indian women, and in tropomyosin α-TMPT. Other research groups in India have reported a few amino acid substitutions in β-MYH7 in patients of Indian descent. However, there is not much data available on the β-MYH7 gene in Indian patients with DCM; therefore, our study will no doubt help in our understanding of the frequency of mutations of the β-MYH7 gene in Indian patients with DCM.

**Limitations**

Genetic testing may also be helpful for diagnosis or clinical management, such as assessing the risk for progressive conduction in a person with DCM. Although we identified novel, unique, and rare mutations in most patients, genotype-to-phenotype correlation within the family varies. Thus, for some patients, the guidelines for DCM recommendations are straightforward. In others, undertaking genetic testing, obtaining their family history, and providing counselling is more complex and problematic. Most of the time, it is difficult to get family samples for genetic analysis. We have failed to get samples in a few families because the patients have not followed the follow-up procedure appropriately and required much counselling involving substantial challenges.

**Conclusion**

In the present study, we detected numerous novel, unique, and rare mutations in the β-MYH7 gene exclusively in patients with DCM (8.0%). Understanding the impact of nsSNPs on protein structure will support therapeutic advances, such as developing several small molecules that may be myosin activators, to rescue cardiac contractility of failing hearts. Therefore, our findings may contribute to understanding of the molecular bases of disease and appropriate diagnosis and thereby help block/reverse/diminish the disease phenotype by either gene editing or promotion of other therapeutic strategies (through personalized medicine).

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Disclosures

The authors have no conflicts of interest to disclose.

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Supplementary Material

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