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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.

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

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*)[8-10,14-16] and *myosin binding protein C* (*MYBPC3*)[8,15,17-19] are the 2 most commonly affected sarcomere genes, of which ∼50% have

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Ethics Statement: The Institutional Ethical Committees (IEC); CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad; CARE Hospitals, Hyderabad, Telangana; and Government Rajaji Hospital, Madurai, Tamil Nadu, approved the study. The blood samples of both patients and controls were collected with informed written consent. 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.

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See page 10 for disclosure information.

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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—Asn676_T-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). 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).

Résultats : L'étude nous a permis de révéler 27 variations, dont sept mutations (8,0 %) étaient exclusivement détectées chez les patients indiens atteints de CMD pour la première fois. Parmi ces mutations, nous avons observé quatre mutations faux-sens—Arg723His, Phe510Leu, His358Leu et Ser384Tyr (2,9 %), une mutation par déphasage—Asn676_T-del (1,5 %) et deux mutations des sites d'épissage (IVS17+2T) T>G et (IVS19-1G) G>A (3,6 %). Étonnamment, les quatre mutations faux-sens changeaient les acides aminés évolutivement conservés. Selon deux outils bioinformatiques—PolyPhen-2 (de l'anglais, *polymorphism phenotyping v2*) et SIFT (de l'anglais, *sorting intolerant from tolerant*), les quatre mutations faux-sens devaient être pathogènes. De plus, les quatre modélisations de β-MYH7 par homologie—p.Leu358, p.Tyr384, p.Leu510 et p.His723—affichaient de façon respective des écarts quadratiques moyens de ∼2,55 Å, ∼1,24 Å, ∼3,36 Å et ∼3,86 Å.
Conclusions : Dans la présente étude, nous avons détecté de nombreuses nouvelles mutations, uniques et rares, dans le gène *β-MYH7*, exclusivement chez les patients indiens atteints de CMD (8,0 %). Ici, nous avons démontré comment chaque mutant (faux-sens) perturbe de manière unique un réseau essentiel d'interactions non liantes au site de mutation (moléculaire) et peut contribuer à la survenue de la CMD. Par conséquent, les conclusions de notre étude peuvent donner un aperçu des bases moléculaires de la maladie et du diagnostic tout en favorisant la promotion de nouvelles stratégies thérapeutiques (par la médecine personnalisée).

accounted for hypertrophic cardiomyopathy,[13,14,20-27] and ∼10% have accounted for DCM.[8-10] Mutations in genes encoding for sarcomeric proteins may contribute to impaired energy metabolism affecting the heart's pumping efficiency.[13,28] The clinical heterogeneity, ranging from asymptomatic to severe conditions such as heart failure and sudden cardiac death in DCM may be due to the change in the heritable component and to environmental influences such as exercise, lifestyle, and gene modifiers.[7,13,20,28,29] Familial DCM is mostly a monogenic disease with an autosomal dominant pattern of inheritance; however, autosomal recessive,[30] X-linked, and mitochondrial inheritances[31] have also been reported.

Although the recent next-generation sequencing technology has significantly increased our knowledge about disease alleles,[32,33] we are still far from having a complete understanding of their impact on disease phenotype, particularly missense mutations. Some missense mutations may affect protein folding and trigger the accumulation of nonfunctional forms of protein such as oligomers and amyloid fibrils and thus cause diseases such as type 2 diabetes, Parkinson's, and prion disease. [34] Protein folding and unfolding are the essential processes for forming its protein structure, and biological activity is dictated by the interactions between their constituent amino acids.[35] In the present genomic era, how a missense mutation can affect its protein 3D structure, stability, and function is still one of the most prominent unanswered questions. Here, in addition to the polymorphism phenotyping 2 (PolyPhen-2) and sorting intolerant from tolerant (SIFT) bioinformatics prediction tools, we used homology models to address, understand, or predict how each missense mutation may contribute to disease pathogenicity. Although hundreds of causative mutations in the *β-MYH7* gene for hypertrophic cardiomyopathy and DCM have been identified in various populations, only a few studies have been conducted on the *β-MYH7* gene in Indian cardiomyopathy patients. Hence, it has remained poorly understood in India. Therefore, we undertook the current study, which is the first comprehensive study of *β-MYH7* in Indian patients with DCM.

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 guidelines[36,37] 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,

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	27.21	10
Dyspnea or shortness of breath	69.2	0
Angina pectoris (chest pain)	56	0
Syncope (fainting)	30	0
Abnormal ECG	68	0
LVEDD, 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; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

history, 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.[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.[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 enzyme, 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 [41]and SIFT.[42] In addition, the homology models were built for all novel missense mutations by SWISS-MODEL homology-modelling (swissmodel.expasy.org).[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)[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 variations 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 Phe510Leu 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 2. Variations detected in β-myosin heavy chain gene (*β-MYH7*) in Indian dilated cardiomyopathy (DCM) patients

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	—	—	—	3	7	Reported
5	1423899060	974488074	C>T	Exon 12	rs735712	G354G	—	—	—	0	6	Reported
6	1423899049	1505811307	A>T	Exon 12	rs606231316	H358L (−1)	Damaging	Damaging	Pathogenic	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	Damaging	Pathogenic	0	1	Novel
9	1423897757	1505811314	C>A	Exon 15	rs606231323	F510L (0)	Damaging	Damaging	Pathogenic	0	1	Novel
10	1423896543	1505811318	T>A	Intron 16	rs606231327	—	—	—	—	0	2	Novel
11	1423896447	1505811320	T>G (IVS17+2T)	Intron 17	rs606231329	SD	—	—	Pathogenic	0	1	Novel
12	1423896337	1505811287	G>A	Intron 17	rs483352953	—	—	—	—	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	Damaging	Pathogenic	0	2	Novel
15	1423896002	342383804	T>C	Exon 18	rs145564868	N676N	—	—	—	0	4	Reported
16	1423895289	4041783	G>C	Exon 19	rs1126421	G682G	—	—	—	6	9	Reported
17	1423895028	1505811326	G>A-(IVS19-1G)	Intron 19	rs606231334	SA	—	—	Pathogenic	0	4	Novel
18	1423895022	1505811293	G>A	Exon 20	rs397516135	R723H (+2)	Damaging	Damaging	Pathogenic	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	Novel
22	1423894161	1505811332	C>T	Exon 22	rs606231339	L832L	—	—	—	0	4	Novel
23	1423894132	1505811299	G>A	Exon 22	rs397516154	E875E	—	—	—	0	3	Reported
24	1423893287	4041786	C>T	Exon 23	rs1041957	A917A	—	—	—	5	7	Reported
25	1423892888	974488065	T>C	Exon 24	rs7157716	I989I	—	—	—	0	4	Reported
26	1423892819	990927921	C>T	Exon 24	rs145379951	A1012A	—	—	—	0	2	Reported
27	1423891240	1505811301	C>G	Intron 25	rs483352965	—	—	—	—	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.

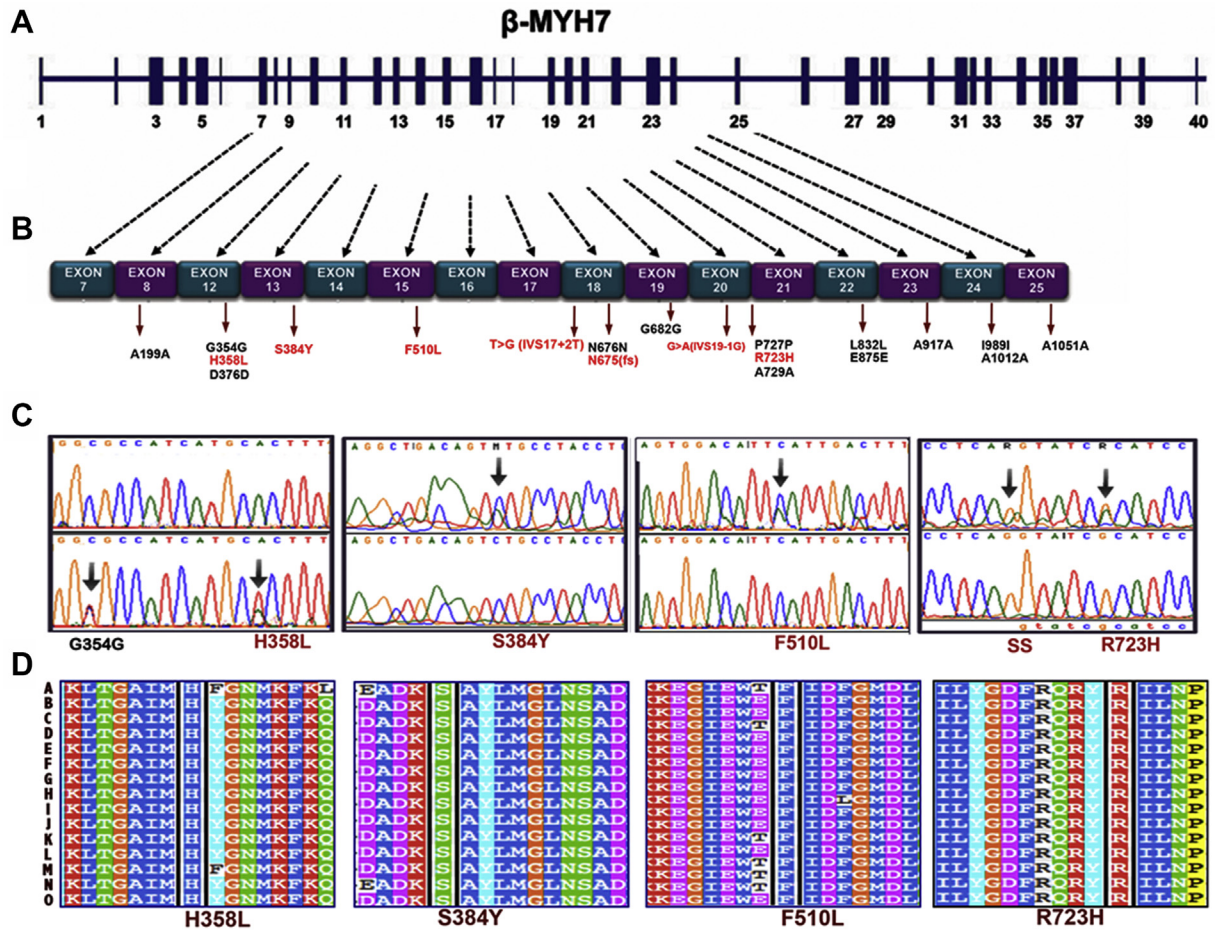

Figure 1. (**A**) Schematic representation of the β-MYH7 structure. (**B**) Highlighted are the observed exonic, splice sites, and frameshift (fs) variations. (The 4 amino acid substitutions, 1 frameshift mutation, and 2 splice-site mutations are indicated in red). (**C**) Electropherograms (arrows) showing 4 missense mutations [CAC→CTC (p.His358Leu), TCT→TAT (p.Ser384Tyr), TTC→TTA (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**: F7HXMO—*Callithrix jacchus* (common marmoset); **D**: P13533—*Homo sapiens* (human); **E**: UPI00022B77DF—*Cavia porcellus* (guinea pig); **F**: G3V885—*Rattus norvegicus* (rat); **G**: P02563—*Rattus norvegicus* (rat); **H**: P13539—*Mesocricetus auratus* (golden hamster); **I**: Q02566—*Mus musculus* (mouse); **J**: Q2TAW4—*Mus musculus* (mouse); **K**: FIN2GO—*Bos taurus* (bovine); **L**: UPI0001D57615—*Bos taurus* (bovine); **M**: F7GOR4—*Macaca mulatta* (rhesus macaque); **N**: UPI0002257BDF—*Canis lupus familiaris* (dog); and **O**: G3RLR1—*Gorilla gorilla gorilla* (Western lowland gorilla).

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 may induce exon skipping, interruption of cryptic splicing sites (CSS), or alternative splicing sites (ALSS), resulting in genetic disorders. In addition, we detected a frameshift mutation [p.Asn676_(T-del)] in exon 18 of the *β-MYH7* gene in a 31-year-old DCM patient with a family history of sudden cardiac death (Fig. 2D). A frameshift mutation may change the reading frame and possibly result in a completely different protein. Therefore, to understand its pathogenicity properly, functional studies need to be carried out.

Remarkably, all 4 missense mutations (nsSNPs)—Arg723His, Phe510Leu, His358Leu, and Ser384Tyr—were predicted to be pathogenic by the PolyPhen-2 and SIFT bioinformatics tools (Table 2). Furthermore, to clearly understand the impact of 4 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 average deviations between the atoms (backbone atoms) of the superimposed pairs (Fig. 3). The RMSDs of the superimposed pairs of each of the 4 (mutants) homology models p.Leu358, p.Tyr384, p.Leu510, and p.His723, of β-MYH7 with the native β-MYH7 template, were ~2.55 Å, ~1.24 Å, ~3.36 Å, and ~3.86 Å, respectively (Fig. 3). We then studied the non-bonding interactions at the mutation site of all 4 homology models of β-MYH7, to understand the mutation (nsSNPs) impact on β-MYH7 structure (Fig. 4). Here, we demonstrated how each mutant (homology model) uniquely disrupts and cause a deviation in a critical network of non-bonding interactions at the mutation site and possibly contributes to disease pathogenicity (Table 3; Fig. 4).

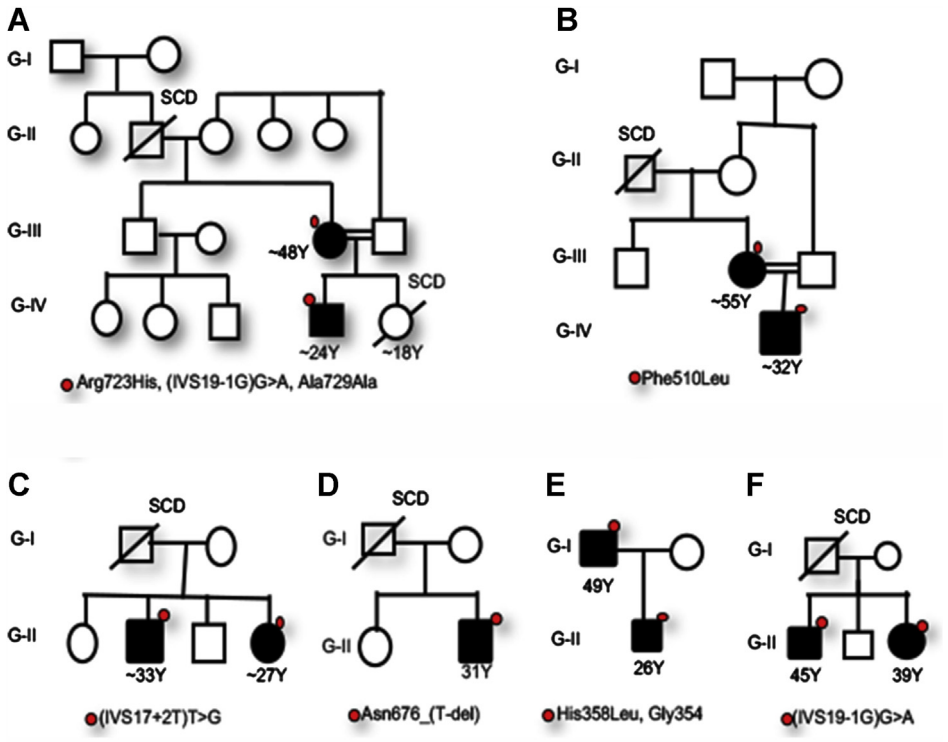

Figure 2. Shown are the pedigrees of dilated cardiomyopathy patients and their family members with novel mutations in the *β-MYH7* gene. G, generation; SCD, sudden cardiac death.

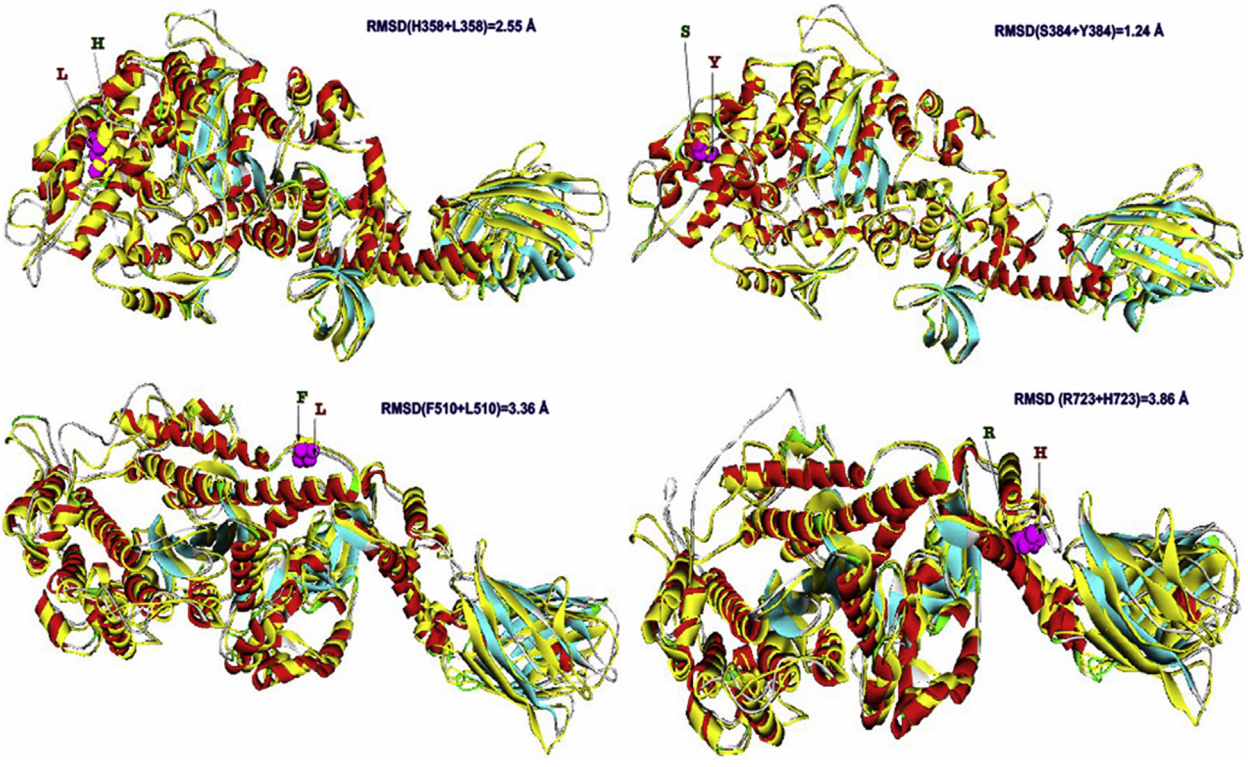

Figure 3. The root mean square deviations (RMSDs) of superimposed 3D structure of each of the 4 mutant homology models with native template. L, leucine; H, histidine; S, serine; Y, tyrosine; F, phenylalanine; R, arginine.

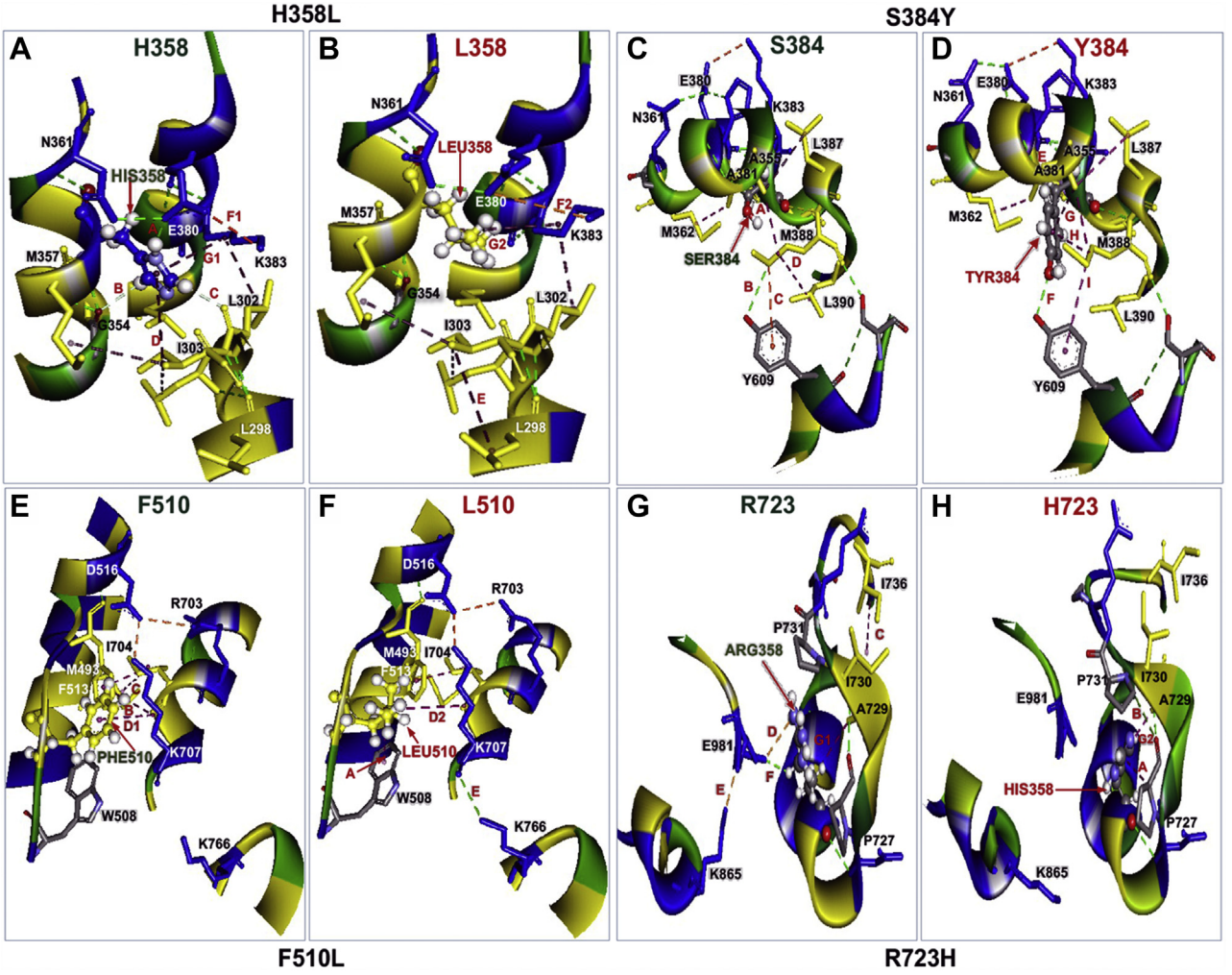

Figure 4. Non-bonding interactions at the site of amino acid substitution in 4 β-myosin mutant homology models vs native template. (**A**) Native His358; (**B**) Mutant Leu358; (**C**) Native Ser384; (**D**) Mutant Tyr384; (**E**) Native Phe510; (**F**) Mutant Leu510; (**G**) Native Arg723; (**H**) Mutant His723.

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

Table 3. The non-bonding interactions of all 4 homology models of β-MYH7 vs native template of β-MYH7 at the mutation site

Amino acid	REF-Non-bond	Distance	labelled	Types	Angle XDA	Angle DAY	Amino acid	MUT-Non-bond	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:HIS358 - A:LYS383	4.16	E1	Pi—Alkyl	—	—		A:LYS383 - A:LEU358	E2	4.76	Pi—Alkyl	—	—
	A:LYS383:NZ - A:GLU380:OE2	3.71	F1	Salt bridge	109	121		A:LYS383:NZ - A:GLU380:OE1	F2	5.13	Salt bridge		
	-	—		—	—	—		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:ALA355 - A:MET388		3.78	Alkyl		
	A:MET388 - A:LEU390	5.09	D	Alkyl	—	—		Bond-destroyed				—	
	NO-BOND	—		—	—			A:TYR384:N - A:ALA381:O	E	3.38	Conventional hydrogen bond	96.1	102
	NO-BOND	—		—	—			A:TYR384:OH - A:TYR609:OH	F	2.62	Conventional hydrogen bond	153	112
	NO-BOND	—		—	—			A:TYR384 - A:ALA355	G	4.35	Pi—Alkyl		
	NO-BOND	—		—	—			A:TYR384 - A:MET388	H	3.79	Pi—Alkyl		
	NO-BOND	—		—	—	—		A:TYR609 - A:MET388	I	5.49	Pi—Alkyl		
PHE510	No-Bond	—		—			LEU510	A:TRP508 - A:LEU510	A	5.08	Pi—Alkyl		
	A:PHE513 - A:LYS707	5.34	B	Pi—Alkyl				Bond-destroyed	—	—	—		
	A:ILE704:N - A:GLU700:O	3.11	—	Conventional hydrogen bond	113	157		A:ILE704:N - A:GLU700:O	—	3.12	Conventional hydrogen bond	113	156
	A:MET493 - A:ILE704	5.46	C	Alkyl				Bond-destroyed	—	—	—		
	A:PHE510 - A:LYS707	4.45	D1	Pi—Alkyl				A:LYS707 - A:LEU510	D2	5.28	Alkyl		
	A:LYS707:NZ - A:ASP516:OD2	2.7	—	Salt bridge	119	128		A:LYS707:NZ - A:ASP516:OD2	—	2.7	Salt bridge	133	126
	A:PHE513 - A:ILE704	4.02	—	Pi—Alkyl				A:PHE513 - A:ILE704	—	4.1	Pi—Alkyl		
	No-Bond	—		—				A:LYS766:NZ - A:LYS707:O	E	3.36	Conventional hydrogen bond	134	131
ARG723	No-Bond	—		—			HIS723	A:PRO727:CA - A:HIS723	A	3.99	Pi—Sigma		
	No-Bond	—		—				A:PRO731:CD - A:PRO727:O	B	3.28	Carbon hydrogen bond		
	A:ILE730 - A:ILE736	4.92	C	Alkyl	158	124		Bond-destroyed	—	—		—	—
	A:ARG723:HH12 - A:GLU981:OE2	3.26	D	Salt bridge; attractive charge	93.3	125		Bond-destroyed	—	—		—	—
	A:LYS865:HZ1 - A:GLU981: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		Bond-destroyed	—	—		—	—
	A:ALA729 - A:ARG723	4.7	G1	Alkyl				A:HIS723 - A:ALA729	G2	3.94	Alkyl		
	A:ARG723:HN - A:ARG719:O	2.1		Conventional hydrogen bond	144	146		A:HIS723:HN - A:ARG719:O		2.23	Conventional hydrogen bond	126	143
	A:ASN726:HN - A:ARG723:O	2.08		Conventional hydrogen bond	145	127		A:ASN726:HN - A:HIS723:O		2.38	Conventional hydrogen bond	120	116

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.[46] 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.[47] Thus, the mutant homology model p.Leu510 of β-MYH7 showed evident RMSDs of ∼3.36Å (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[41] and SIFT,[42] 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 ∼1.24Å° (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.[35] 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.[48] 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.[34]

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*),[17] troponin I3 (*TNNI3*),[39,49] troponin T2 (*TNNT2*),[40,50] actin (*ACTC*),[51] and myosin (*β-MYH7)* in Indian women,[52] and in tropomyosin *α-TMP1.*[53] Other research groups in India have reported a few amino acid substitutions in *β-MYH7* in patients of Indian descent.[54-56] 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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