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Research Article

A Missense Mutation (R723H) in the Head mOtor Domain of β-MYH7 gene in an Indian HCM Patient and Phenotypic Plasticity

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Abstract

Mutations in the β-MYH7 gene are one of the major causes that lead to cardiomyopathies. However, to differentiate a causative nsSNP and its impact on protein structure remains a major challenge. In the present study, we detected a missense mutation Arg723His in the head motor domain of β-MYH7 in a HCM patient, and it was absent in 207 healthy individuals. The mutant (R723H) has been found to alter an evolutionarily conserved amino acid. In addition, the mutant (R723H) was predicted pathogenic by Polyphen-2 and SIFT bioinformatic tools. Further, the superimposed 3D structure of the mutant (p.His723 homology model) with native (p. Arg723) displayed the root means square deviation (RMSD) of ~3.38A0. We know that the non-covalent interactions such as hydrophobic, electrostatic, Van der Waals, and hydrogen bonds between amino acids are at the heart of stabilizing protein structures. Here, we demonstrated how the mutant (p.His723) has disrupted a critical non-covalent interactions network at the mutation site and may contribute to the disease phenotype. Hence, our findings in the future could pave the way for developing small molecular modulators or myosin-targeted therapies for heart failure.

Key words: β-myh7; cardiomyopathy; homology models; 3d structure; sarcomere genes; hcm; dcm

Introduction

In general, non-synonymous Single Nucleotide Polymorphisms (nsSNPs) lead to change an amino acid of the encoded protein. They may affect protein structure, stability, and function, which may cause various diseases in humans [1-4]. About ≥50% of amino acids change are linked with genetic disorders [5,6]. However, a few amino acids change have remained uncharacterized in genes [7]. Mutations in the sarcomere proteins were reported to cause cardiomyopathies in various populations [8-15]. However, identifying the causative nsSNPs and their association led to disease is challenging. Cardiomyopathy is classified into Hypertrophic (HCM) and dilated (DCM) based on their heart muscle structure[16]. The HCM is characterized by excessive left ventricular thickening, arrhythmia, diastolic dysfunction, left ventricular outflow obstruction, myocardial ischemia, mitral regurgitation, and sudden death, with an estimated prevalence of 1:500[17]. Mutations in sarcomere genes predominantly cause HCM, of which ~75% of mutations were reported in the *β-MYH7* and *MYBPC3* genes [6,18-26]. Though the recent next-generation sequencing (NGS) technology has significantly increased our knowledge about disease alleles [27,28], we are far from completely understanding the impact of deleterious alleles on disease phenotype. We know that a few mutations may lead to a misfolding and nonfunctional form of proteins to accumulate and which may cause diseases. More importantly, the interactions between constituent amino acids in a protein determine its 3D structure and function [29]. Here, we have demonstrated a deleterious effect of a missense mutation (Arg723His) on β-MYH7 protein structure using (p.His723) homology modeling, an *Insilco* analysis.

Materials and Methods

Ethical statement and clinical evaluation

We enrolled a total of 50 hypertrophic cardiomyopathy patients (HCM) from 2 hospitals (Table 1). They were (a) Baba Clinical and Genomic

Research Centre, CSIR Road, Taramani, Chennai, India, and (b) Academics and Research, Global Hospitals and Health City, Chennai, India. Along with 207 healthy volunteers matched for the age, sex and ethnicity were recruited as controls (Table 1), provided they had normal ECG and echocardiograph measurements and were unrelated to the HCM patients. The Institutional Ethical Committees (IEC) of all three institutes have approved the study. Before the sample collection, informed written consent was obtained from all patients and controls to fulfill the requirements of relevant guidelines and regulations that permitted research on human subjects, which has followed the ethics of the Declaration of Helsinki, the World Medical Association.

Baseline characteristics	HCM (N=50)	Controls (N=207)
Age (Yrs)	49±12	50.0 ±0.2
Sex, males (%)	61	63
Dyspnea or shortness of breath %	67	0
Angina pectoris (chest pain) %	56.8	0
Syncope (fainting) %	31.9	0
Abnormal ECG %	57.4	0
LVEDD, mm	36± 6.5	51.3± 2.7
LVESD, mm	20.8 ± 3.7	32.1± 1.2
Septum, mm	22.1 ± 4.2	9.0 ± 0.2
Family History %	37	0
Sudden cardiac death %	21.8	0

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.

Table 1: *Clinical baseline characteristics of HCM patients with control*

Genetic studies

The patients and controls DNA was extracted from peripheral blood samples, amplified using polymerase chain reaction (PCR), as described elsewhere [13]. The amplicons were purified using Exonuclease 1 and Shrimp alkaline phosphatase, following the manufacturer's instructions (USB Corporation, 26, 111 Miles Road, Cleveland, Ohio 44128, USA). The purified amplicons were sequenced bi-directionally using the ABI Big Dye terminator cycle sequencing kit (Perkin–Elmer, Foster City, CA, USA) and ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Using Auto-Assembler software from Applied Biosystems (Foster City, CA, USA), the sequences were edited and screened for variations compared with the respective reference sequence obtained from Gen-Bank.

In silico analyses

A nonsynonymous single-nucleotide variant observed in our study was analysed using two bioinformatics tools, PolyPhen-2 (Polymorphism Phenotyping v2; http://genetics.bwh.harvard.edu/pph2/) [30] and SIFT (Sorting Intolerant From Tolerant; http://siftdna.org/www/Extended_SIFT_chr_coords_ submit.html). [31] Further, we built a homology model for a mutant of *β-MYH7* by SWISS-MODEL Repository System (SMTL) (http://swissmodel.expasy.org) [32], using 3D native template structure having 99% similarity obtained from the RCSB protein data bank (PDB) (http://www.rcsb.org/pdb/explore/explore.do?structureId=4P7H) [33]. To understand the impact of a nsSNP on β-MYH7 protein structure, we first superimposed the homology model of β-MYH7 with native β-MYH7 protein template structure to measure their root-mean-square deviations (RMSD) between the atoms (backbone atoms) of the superimposed pairs. We second studied the non-bonding interactions (created/destroyed) at the mutation site of the homology model vs. native *β-MYH7*. We then plotted the hydrophobicity plot and Ramachandran plot and studied the homology model vs. native *β-MYH7*.

Results

In the present study, we detected a missense mutation (R723H) in the head motor domain of *β-MYH7* (Fig.1A). We found that the mutant (R723H) has altered the evolutionarily conserved amino acid across many species (**Figure 1A, B**). The mutant (p. His723) was predicted pathogenic by Polyphen2 and SIFT bioinformatics tools. Further, to understand the impact of mutant (Arg723His) on its protein structure, we first superimposed the mutant (p.His723_homology model) with native β-MYH7 protein (p.Arg723) and measured their root-mean-square deviation (RMSD), it was ~3.86A^{o}.

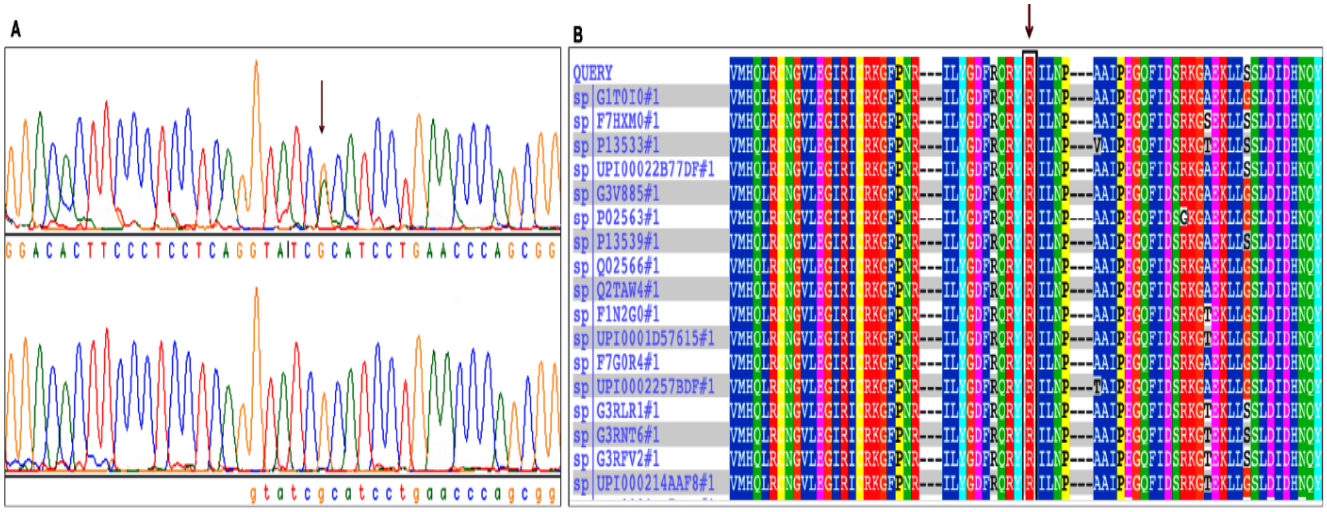

Figure 1A: *Electropherograms (arrows) showing a missense mutation CGC→CAC (p.Arg723His), in the β-MYH7 gene.*
Figure 1A: *Multiple alignments of amino acid sequences in the β-MYH7 gene of several species, showing that the amino acid p.Arg723 is highly conserved across many species*

We then compared the non-bonding interactions of a homology model (p.His723) vs. native β-MYH7 protein to understand the mutational impact on protein structure and function (**Table 2; Figure 2**). Here, we observed that the mutant His723 forms a peculiar hydrophobic interaction with Pro727 (A), which, in turn, forms a hydrogen bond with another nearby proline residue Pro731 (B). As a result, a hydrophobic interaction between two isoleucine residues (Ile730 and Ile736) has been destroyed (C) due to an increased van der Waals radius (**Table 2; Figure 2**). Further, the mutant p.His723 also destroys two electrostatic salt bridges; Arg723 with Glu981 (D) and Glu981 with Lys865 (E) (**Table 2; Figure 2**). We know that the proline residue is unique and lacks an amide proton; therefore, it can't donate hydrogen to stabilize other bonds or promote stability, thus possibly making the mutant structure very rigid. The deviations in the mutant could be clearly understood when we compare the hydrophobic interaction distances between the native Arg723 with Ala729 (4.69 A^0) in the template (F1) and the mutant His723 with Ala729 (3.93 A^0) in the homology model (F2) (**Table 2; Figure 2**).

Non-bonding Native_ARG723	Distance	Non-bonding Mutant_HIS723	Distance	Types	Labelled	Angle XDA	Angle DAY	Angle XDA	Angle DAY
A:ARG723:HH12 - A:GLU981:OE2	3.25737	Bond-destroyed	-	Salt Bridge-Attractive Charge	D	93.26	125.02	-	-
A:ARG723:HN - A:ARG719:O	2.09792	A:HIS723:HN - A:ARG719:O	2.23	Conventional Hydrogen Bond	-	144.1	146.15	126	143.34
A:ASN726:HN - A:ARG723:O	2.08037	A:ASN726:HN - A:HIS723:O	2.38	Conventional Hydrogen Bond	-	145.5	126.62	120	115.67
A:ALA729 - A:ARG723	4.69552 (F1)	A:HIS723 - A:ALA729	3.94 (F2)	Alkyl-Hydrophobic	F1&F2	-	-	-	-
A:LYS865:HZ1 - A:GLU981:OE1	2.42839	Bond-destroyed	-	Salt Bridge-Attractive Charge	E	132.1	114.64	-	-
NO-BOND	-	A:ILE730:HN - A:HIS723:NE2	2.73	Conventional Hydrogen Bond	-	157.9	123.92	136	126.46
A:ILE730:HN - A:PRO727:O	2.21757	A:ILE730:HN - A:PRO727:O	2.44	Conventional Hydrogen Bond	-	-	-	129	108.4
A:ILE730 - A:ILE736	4.91767	Bond-destroyed	-	Alkyl-Hydrophobic	C	157.9	123.92	-	-
A:ILE730:HN - A:PRO727:O	2.21757	Bond-destroyed	-	Conventional Hydrogen Bond	-	-	-	-	-
NO-BOND	-	A:PRO727:CA - A:HIS723	3.99	Pi-Sigma	A	-	-	-	-
NO-BOND	-	A:PRO731:CD - A:PRO727:O	3.28	Carbon Hydrogen Bond	B	-	-	-	-

Table 2: *The non-bonding interactions of a homology model p.His723 Vs. native template p.Arg723 of β-MYH7 at the mutation site*

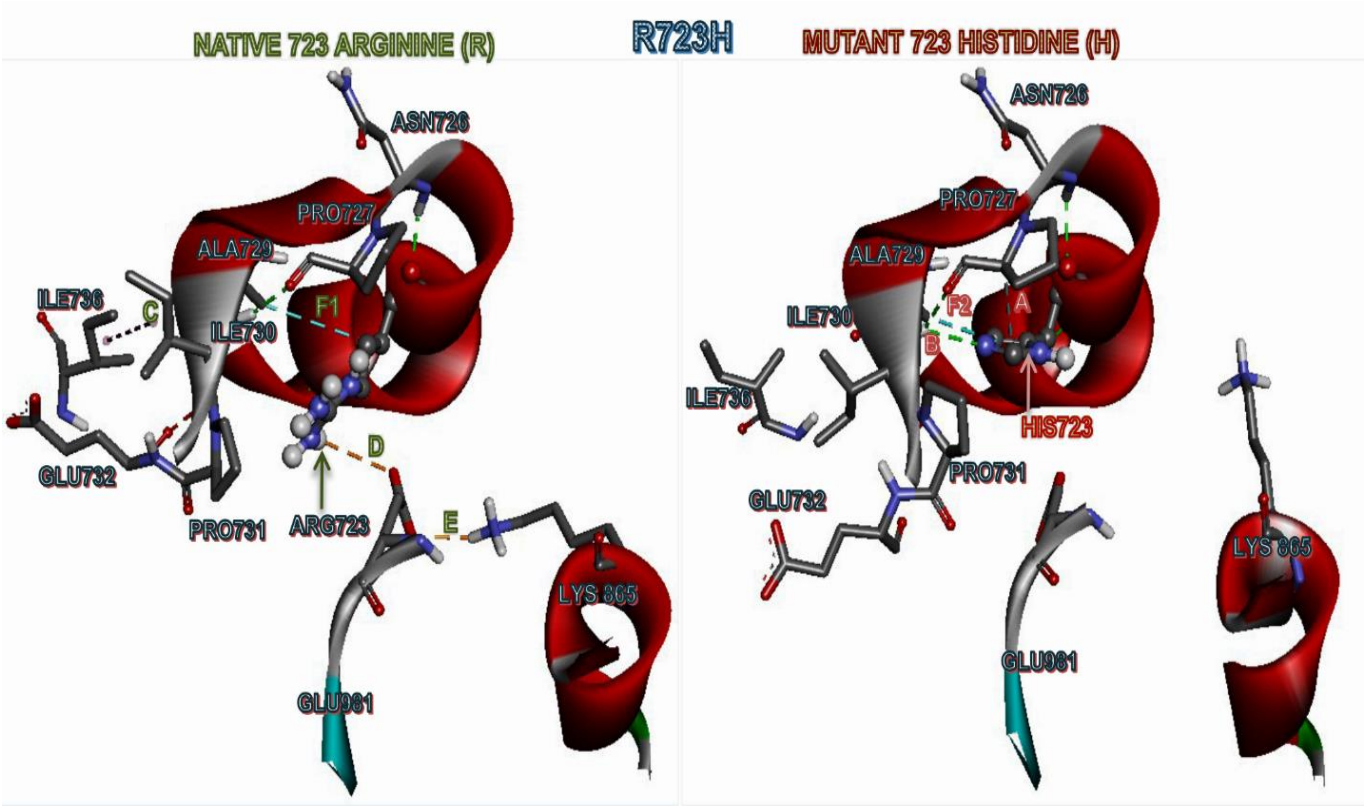

Figure 2: *Non-bonding (NB) interactions at the site of amino acid substitution in a β-myosin mutant homology models vs. native template.*

We plotted the hydrophobicity plot to compare the hydrophobicity index of native protein Vs. mutant protein (**Figure 3; Table S1**).

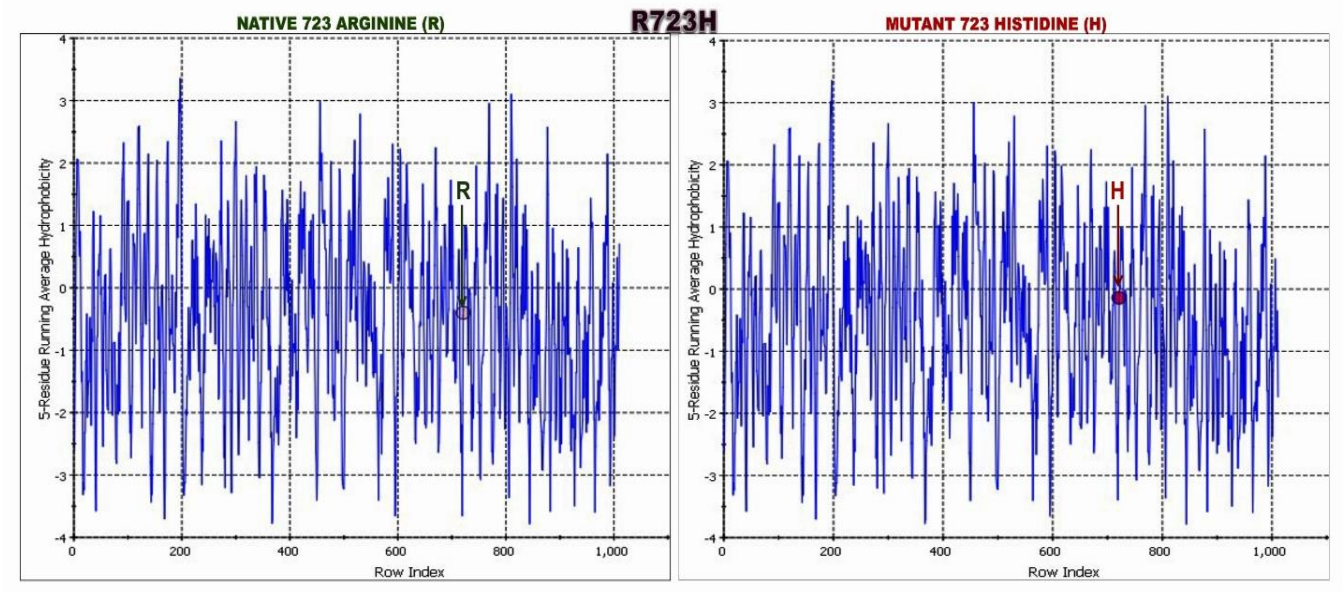

Figure 3: *Hydrophobicity plot, we compared the amino acids in the native protein Vs mutant protein against their hydrophobicity index*

We then studied the Ramachandran plot to compare the energetically allowed and disallowed regions of backbone dihedral angles ψ against ϕ of amino acid residues in the mutant (homology model) vs. native (**Figure.4; Table S2**).

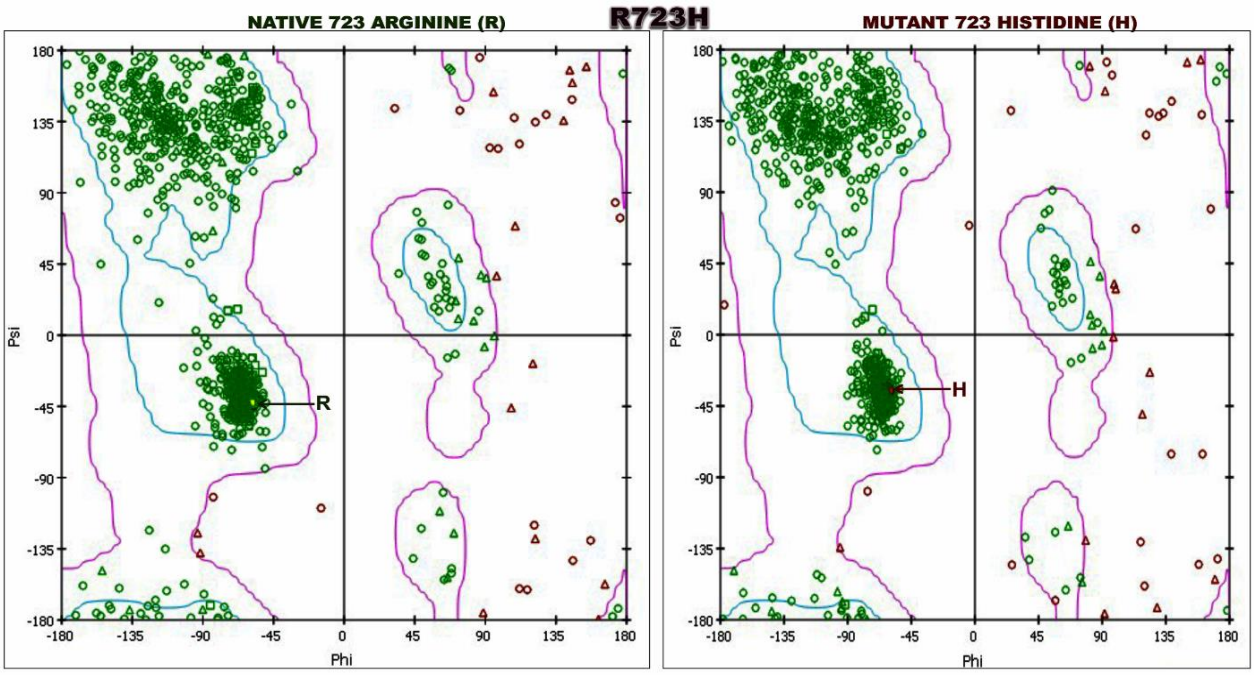

Figure 4: *Ramachandran Plot, we compared the energetically allowed and disallowed regions of backbone dihedral angles ψ against ϕ of amino acid residues in the homology model vs. native.*

Discussion

Mutations in a gene can be benign or pathogenic, and it is often challenging to establish which variants are pathogenic. The *MYH7* is reported to be an important gene, and its mutations may lead to both HCM as well as DCM pathogenesis. Therefore, it is difficult to understand how a mutation can contribute to disease onset and how a gene mutation causes diverse cardiomyopathy phenotypes. In the present study, we found a nsSNP (Arg723His) in the head motor domain of β-MYH7 in a HCM patient (Figure.1A). However, we previously reported the same missense mutation Arg723His along with two other variations [(IVS19-1G) G>A, Ala729Ala] in exon 20 of the *β-MYH7* gene (allelic heterogeneity) in a DCM patient and her son [34] [Rani et al 2021]. Thus, strongly reinforcing phenotypic plasticity in the presence of the compound mutation besides environmental background, epigenetic modifications/other factors, etc., [35,36]. The role of epidemiological factors in the pathogenetic process gains even more prominence because a single mutation can sometimes give rise to two very divergent phenotypes, emphasizing the role of gene modifiers and the influence of environmental factors in accounting for phenotypic plasticity [37,38]. This missense mutation R723H was absent in 207 healthy individuals. The mutant p.His723 has been found to alter evolutionarily conserved amino acids. It was predicted pathogenic by Polyphen-2 and SIFT bioinformatic tools. Further, we found that the mutant p. His723 (homology model) uniquely disrupts and deviates a critical network of non-bonding interactions at the mutation site (Table2; Fig.2). We know that a network of different kinds of non-covalent interactions between the amino acid residues drive the accurate 3D structure of the protein. Though different kinds of molecular interactions determine the accurate 3D structure of the protein, a network of non-covalent interactions between them is crucial [39]. We showed hydrophobicity plot (Figure.3; Table S1) and the Ramachandran plot (Figure.4; Table S2) to understand the deviation in mutant protein structure. Though different sequences map to a similar structure, a nsSNP can dramatically change a protein structure and lead to disease phenotype, such as sickle cell anemia (glutamic acid to valine (E6V) in the β-globin) [40]. Some studies suggest that the abnormal proteins themselves serve as pathogenic agents and are associated with various diseases [41]. However, functional studies are needed to confirm the actual pathogenic effect of this mutation.

Conclusion

Here, we have demonstrated how the (p.His723) mutant disrupted a critical non-covalent interactions network that possibly affects the structure and function. Therefore, understanding the impact of nsSNP on protein structure is indispensable for targeting the mutant amino acid residue for therapeutic purposes. Our findings in future could pave the way for developing small molecular modulators or myosin-targeted therapies for failing hearts.

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Conflict of Interest

The authors have declared that there is no conflict of interest exist.

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Author Contributions

D.S.R. and K.T. conceived and designed the experiments. D.S.R performed PCR, Direct sequencing and mutation screening. D.S.R has done RMSD and Non-bonding interaction *Insilico* analysis with technical help from A.S. Cases and control samples were received from G.V.S., and E.C.

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