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Original Article

Mutation in the beta-myosin heavy chain *(β-MHC)* gene of adult Bangladeshi patients with hypertrophic cardiomyopathy

Laila Anjuman Banu[1], Md. Mohiuddin Masum[1], Susmita Rahman[1], Sultana Mahbuba[1], Mahmud Hosasain[2], Mohammad Jakir Hosen[3], Toufiq Ahmed[4], Sajal Krishna Banerjee[5], Dipal Krisna Adhikary[5], SM Ahsan Habib[5], Gazi Nurun Nahar Sultana[6], M Nazrul Islam[5]

[1]Department of Anatomy, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[2]Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka, Bangladesh
[3]Department of Genetic Engineering & Biotechnology, Shahjalal University of Science & Technology, Sylhet, Bangladesh
[4]Department of Internal Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[5]Department of Cardiology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[6]Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka, Bangladesh

Correspondence to: Professor Laila Anjuman Banu, Email: dr.lailabanu@bsmmu.edu.bd

Abstract

Hypertrophic cardiomyopathy (HCM) is the most prevalent genetic cardiomyopathy characterized by sudden cardiac death. HCM is caused by the mutation in several genes that encode sarcomere proteins. Beta-Myosin Heavy Chain (*β-MHC)* gene is the one of the most mutated genes responsible for HCM. Studies on mutation spectrum of β-MHC gene are lacking in the Asian population including Bangladeshi patients. This study was intended to mutational analysis of β-MHC gene in Bangladeshi HCM patients. A cross-sectional study was conducted for mutation analysis of the *β-MHC* gene on 70 Bengali Bangladeshi HCM probands using next-generation sequencing at the Genetic Research Lab of Bangabandhu Sheikh Mujib Medical University. Structural and functional impact of the mutations were further analyzed by in-silico process. Thirty-nine nucleotide variants were found in both exonic (36%, n= 14) and intronic regions (64%, n=25) of *β-MHC* gene. We found 14 missense mutations, including the p.Glu965Lys, p.Arg941Pro, p.Lys940Met, p.Glu935Lys, and p.Met922Lys that are associated with inherited HCM. Most variants were heterozygous and one homozygous (p.Val919Leu) was found. The variant with most evidence of causing the disease was p.Glu935Lys. Among the missense variants, nine were not noted in ClinVar, dbSNP, GenomeAD databases. These unreported variants located between myosin head and tail domains might be novel mutations for Bangladeshi population. We found nine novel variants in the *β-MHC* gene. Findings of this research will help to developing a genetic database of HCM for early diagnosis and proper management of HCM patients in Bangladesh.

Keywords: *Hypertrophic cardiomyopathy, genetics, mutation, β-MHC, Bangladeshi*

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the leading cause of sudden cardiac death among young individuals and young athletes.[1] It is also the most common cardiomyopathy with a prevalence of 1 in 500 and is recognized as a global disease.[2-3] HCM has already been reported in 122 countries and approximately 20 million people have been affected all over the world.[4] The clinical features of HCM are heterogeneous, predominantly characterized by left ventricle hypertrophy, diastolic dysfunction, and increased ventricular arrhythmia.[5] It is an autosomal-dominant genetic disorder mainly caused by mutations in sarcomere proteins encoding genes, which accounts for the cause of over 95% of all HCM cases.[6] However, other reasons include mitochondrial cytopathies, carnitine deficiency, the disorder of fatty acid metabolism, and syndromes such as Noonan syndrome, LEOPARD syndrome, and Friedreich ataxia.[7]

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Highlights

1. **This is the first HCM related genetic study of the β-MHC gene in Bangladesh using NGS technology.**
2. **We have identified nine potential novel mutations in the β-MHC gene.**
3. **This may provide basis for further advancement of genetic diversity of the β-MHC gene in Bangladeshi people.**

Over 1400 mutations in 11 or more genes encoding the sarcomere proteins have already been described as the cause of HCM in the different populations.[8] Among the sarcomere proteins encoding genes, *β-MHC (MYH7), MYBPC3, TNNT2,* and *TNNI3* are the most frequently associated with HCM.[9] The *β MHC* gene is the first gene responsible for the genetic cause of HCM.[10] The *β MHC* gene is composed of 40 exons, 38 of which are coding that encompasses around 23kb of DNA.[11] The cross bridging or interaction of the β myosin head with actin is accountable for muscle contraction.[12] The HCM frequency associated with *β-MHC* gene mutations is 30% while 2-7% in the population of a South Asian country, India.[8,13]

Patients with HCM are mostly diagnosed with a routine examination. Echocardiography is the most reliable diagnostic modality for diagnosing HCM.[14] However, genetic testing has become popular for precise diagnosis and treatment. There is paucity in the information about the gene mutations in HCM in the south-Asian populations. Around 4% of HCM cases were reported in Bangladesh where the patients had never previously been diagnosed with cardiovascular diseases, and the genetic testing is yet to start.[15] This is the first study on the genetic analysis on the HCM patients. Thus, this study aimed to the variant screening and mutational analysis of *β-MHC* gene from Bangladeshi HCM patients. This will help in the underlying genetic cause and prognosis of this disease in the Bangladeshi population.

METHODS

Study design and study site

This study was a descriptive, cross-sectional study was done in the Department of Anatomy, Bangabandhu Sheikh Mujib Medical University (BSMMU) from 2016 to 2019.

Sampling technique and sample size

A convenient sampling technique was applied. Seventy adult HCM patients of either sex who were Bengali by ethnicity, Bangladeshi by residence were selected for this purpose. After preliminary selection of HCM patients from various hospitals, clinics and private practice cases, a team of expert cardiologists verified their diagnostic reports. Informed written consent was obtained from each the patients. The patients were selected according to a set of echocardiographic criteria (echocardiographic LV wall thickness ≥ 15mm in any segment of the myocardium).[16] Patients with vulvular disease, coarctation of aorta, hypertension, diagnosed metabolic disorder and myocardial infiltrative diseases were excluded.

DNA extraction and next-generation sequencing

Three ml of blood was obtained from each patient and was taken into tubes containing EDTA (1 mg/ml). Genomic DNA was extracted from the lymphocytes of the peripheral blood samples using a standard blood DNA isolation kit (Promega, Wisconsin, USA). Concentration and quality of the extracted DNA was measured by using a spectrophotometer.

Then 50 ng of genomic DNA was taken for sequencing. The genomic DNA was simultaneously fragmented and tagged to convert it into adapter-tagged libraries. The pooled libraries were hybridized with biotinylated probes. The pool was then enriched with streptavidin beads that bind to the biotinylated probes. The enriched sample pool was added to the Miseq flow cell for sequencing. During the sequencing step of the clusters of DNA fragment were sequenced by simultaneous synthesis and sequencing. Sequencing is based on fluorophore-labelled deoxy nucleotide triphosphates (dNTPs) with reversible terminator elements that became incorporated and excited by a laser one at a time. After reading the forward DNA strand, the reads were washed away, and the process repeats for the reverse strand. The actual raw data of sequencing were images, but they were converted to base calls.

Data analysis

Sequenced raw data was then analyzed using Miseq reporter software "Variant studio". This software aggregates information from multiple sources into a single, maintained database which captures annotations

at variant, gene, and transcript levels. For the comprehensive analysis and interpretation of variant data, these VCF files were subsequently analyzed. Variant Effect Predictor (VEP)[17] is a central resource for thorough annotation of transcript consequences. VEP also leverages databases such as NCBI Reference Sequence Database (RefSeq)[18] and algorithms such as Polymorphism Phenotyping (PolyPhen)[19] and SIFT[20].

RESULTS

A total 39 nucleotide variants were found in the *β-MHC* gene from 70 HCM patients. Variants were found both in exonic ad intronic region. Among these variants, 36% (n=14) were in the intronic region and 64% (n=26) were in exonic regions **(Figure 1).** Nucleotide variants in the *β-MHC* gene includes single nucleotide variants (SNV), insertion, and deletion.).

Transcriptomic analyses of the exonic region were also done. Variants in the coding region, the maximum frequency was observed in missense substitution (36%, n= 14). Synonymous alterations were identified in 9 (23%) of all reported variants. Two in frame insertion (5%) were identified **(Figure 1).**

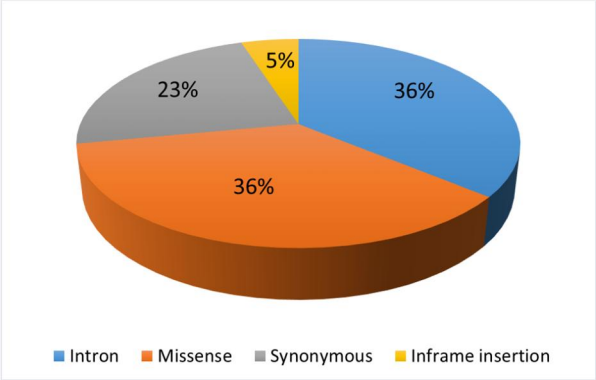

FIGURE 1 The involvement of different *β-MHC* gene variants

By analyzing the distribution of the nucleotide variants on the exon and intron, the intronic variants were in intron 7, 16, 17, 18, 19, and 39 **(Figure 2).** Besides one upstream deletion was also identified in the intronic region.

We found nucleotide alteration in two of the forty exons of the *β-MHC* gene. All the identified missense substitutions were found in the exon 18 and the synonymous alterations were in exon 17 and 18 **(Figure 2)**. However, one in frame insertion was in the exon 18 **(Figure 2).** The in frame insertion (p.Val919_Lys920insThr) was heterozygous and likely pathogenic according to ClinVar.

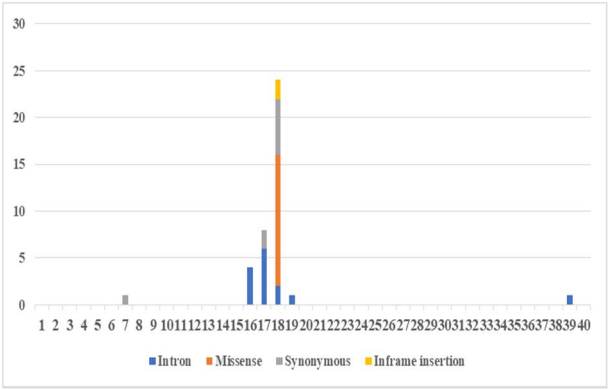

FIGURE 2 Distribution of the nucleotide variants on the exon and intron of *β-MHC* gene

Results regarding the clinical significance of the mutation.

Missense substitutions were examined to identify the variants that could be considered pathogenic in the study population. Then, the variants that were located on the exons and resulted in alterations to the amino acids in the *β-MHC* protein were filtered. We found 14 missense alterations, including the p.Glu965Lys, p.Arg941Pro, p.Lys940Met, p.Glu935Lys, and p.Met922Lys that are already known be associated with inherited hypertrophic cardiomyopathy.

Most of the variants were in heterozygous state and only one homozygous condition (p.Val919Leu) was found **(Table 1 and 2)**. The results of variant pathogenicity on the databases and in silico analysis are presented in **Table 1** (disease causing variant) and in **Table 2** (variants possibly novel).

The results from various sources were not always consistent, as seen in the table, and contradicting results were noted. Variant with most evidence of causing disease was p.Glu935Lys. However, among the missense variants ten variants were not noted in ClinVar, dbSNP or GenomeAD databases. These unreported variants are located in between the myosin head domain and myosin tail domain **(Figure 3)**.

TABLE 1 Disease causing variant

Nucleotide position	Variant	Geno type	HGVSc	HGVSp	ClinVar Significance	Sift	Polyphen
23893145	C>C/T	Het	2893G>A	Glu965Lys	Likely pathogenic	Deleterious	Probably damaging
23893216	C>C/G	Het	2822G>C	Arg941Pro	Likely pathogenic	Deleterious	Probably damaging
23893219	T>T/A	Het	2819A>T	Lys940Met	Likely pathogenic	Deleterious	Probably damaging
23893235	C>C/T	Het	2803G>A	Glu935Lys	Pathogenic	Deleterious	Possibly damaging
23893273	A>A/T	Het	2765T>A	Met922Lys	Pathogenic	Deleterious	Probably damaging

Polyphen= Polymorphism Phenotyping
Sift= Sorting intolerant from tolerant
HGVSp= HGVS protein
HGVSc= HGVS coding sequence

DISCUSSION

The most prevalent hereditary heart disease, HCM exhibits significant clinical and genetic variation. The diagnosis and categorization of at-risk family members who need routine clinical follow-up can be affected by accurately assessing the pathogenicity of discovered variations. After evaluation of the *β-MHC* gene variants,14 missense variants were found and among those variants 10 could be related with the development of HCM.

The missense mutation in the 23893145-nucleotide position (according to the Genome Reference Consortium Human Build 37- GRCh37)[21] which changes the results in changes in the amino glutamic acid to lysine at 965 position of the beta myosin heavy chain protein. According to dbSNp and Clinvar it is a likely pathogenic single nucleotide variant that is responsible for inherited HCM.[22-23] Two reported variation found in 23893216 position where one is variant of uncertain significance (Arg941His) and another one in likely pathogenic variant (Arg941Pro) that we have found in our study.[24-25] Another variant at 23893219 position causes replacement of lysine at 940 position with methionine and likely pathogenic to development of HCM.[26] Two pathogenic variants were identified at 23893235 and 23893273 position where lysine replace glutamic acid and methione at 935 and 922 position of the beta myosin heavy chain protein respectively.[27-28]

TABLE 2 Variant possibly novel

Nucleotide position	Variant	Geno type	HGVSc	HGVSp	ClinVar Significance	Sift	Polyphen
23893136	T>T/G	Het	2902A>C	Lys968Gln	Not listed	Deleterious	Probably damaging
23893141	T>T/A	Het	2897A>T	Lys966Met	Not listed	Deleterious	Probably damaging
23893142	T>T/G	Het	2896A>C	Lys966Gln	Not listed	Deleterious	Probably damaging
23893228	G>G/A	Het	2810C>T	Thr937Ile	Not listed	Deleterious	Possibly damaging
23893232	G>G/T	Het	2806C>A	Leu936Ile	Not listed	Deleterious	Benign
23893242	C>C/G	Het	2796G>C	Met932Ile	Not listed	Tolerated	Benign
23893248	C>C/A	Het	2790G>T	Glu930Asp	Not listed	Deleterious	Probably damaging
23893283	C>C/G	Het	2755G>C	Val919Leu	Not listed	Tolerated	Benign
23893283	C>G/G	Hom	2755G>C	Val919Leu	Not listed	Tolerated	Benign

Polyphen= Polymorphism Phenotyping
Sift= Sorting intolerant from tolerant
HGVSp= HGVS protein
HGVSc= HGVS coding sequence

However, we also found nine missense single nucleotide variants that were not noted in the ClinVar, dbSNP or GenomeAD databases could and possibly novel mutation for the HCM patients. The clarification of novel missense variants with clinical significance is a

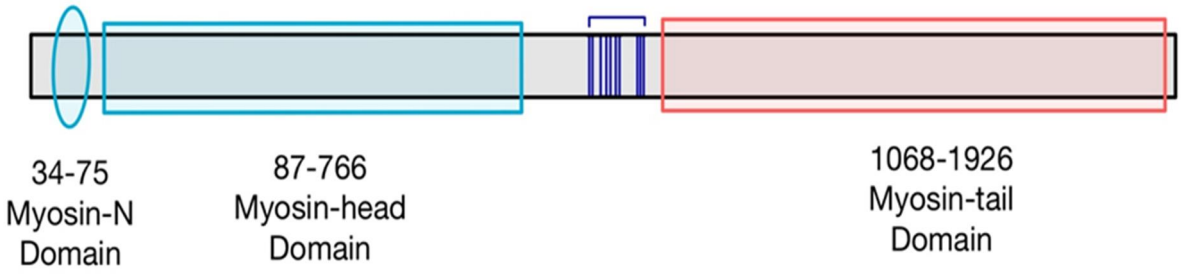

FIGURE 3 The location of the ten unreported variants is shown on the MyHC protein.

challenging and therefore, we performed various in silico bioinformatic analyses using sing SIFT and PolyPhen to predict to predict the likely pathogenicity of missense variants.[29] These unreported variants need further analysis to be confirmed as pathogenic novel mutation.

Major strength of our study is successfully sequencing the entire 22,883 bp sequence of the *β-MHC* gene using the next-generation sequencing technology where we have found both intronic and exonic variants. The findings from this study will aid in the development of mutational database on HCM that will way out the diagnosis of the HCM. Though we have found several mutations, but it was not possible to draw genotype-phenotype correlation as this this requires years of follow-up and mutation analysis of HCM patients as well as their family members.

Conclusion

This is the first study on the mutational analysis on the HCM patients in Bangladesh involving the whole gene sequencing using the next-generation sequencing technology. In this study, we found ten novel variants in the *β-MHC* gene. Findings of this research will help to develop a genetic database of HCM in Bangladesh which will help in early diagnosis and proper management of HCM patients in Bangladesh. By using next-generation sequencing technology, we were able to perform whole gene sequencing, which will enable early diagnosis and targeted sequence analysis for Bangladeshi HCM patients. As a result, this will facilitate proper management of HCM patients in Bangladesh.

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Author Contributions

- Conception and design: LAB, GNNS, MNI
- Acquisition, analysis, and interpretation of data: LAB, MMM, MS, SR, MH, MJH, SKB, DKP, SMHA
- Manuscript drafting and revising it critically: LAB, MMM
- Approval of the final version of manuscript: LAB, GNNS, MNI
- Guarantor accuracy and integrity of the work: LAB, GNNS, MNI

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Conflict of Interest

None

Ethics approval

This research was approved by the Institutional Review Board (IRB) of BSMMU (No: BSMMU/2014/3531; Date: 20.03.2014).

ORCID iD:

Laila Anjumab Banu, https://orcid.org/0000-0003-4409-9009

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