

# Study of Clinical Profile of HCM Patients Carrying 25 BP Deletion in Intron 32 of Cardiac Myosin Binding Protein C (MYBPC3) Gene

Dr. Sagar Makode<sup>1</sup>, Dr. Ajay Bahl<sup>2</sup>, Madhu Khullar<sup>3</sup>, Dr. Saurabh Mehrotra<sup>4</sup>, Dr Rutuja Darokar<sup>5</sup>

<sup>1</sup>Assistant Professor, AIIMS, Nagpur (Ex Senior resident, PGIMER, Chandigarh)

<sup>2</sup>Professor, Department of Cardiology, PGIMER, Chandigarh

<sup>3</sup>Professor, Department of Experimental Medicine and Biotechnology

<sup>4</sup>Additional Professor, Department of Cardiology, PGIMER, Chandigarh

<sup>5</sup>Senior Resident, AIIMS, Nagpur

**Abstract:** *The present study aimed to study the frequency of “25 BP” deletion in intron 32 of “MYBPC3” gene along with clinical characteristics of the Hypertrophic Cardiomyopathy patients carrying for this mutation in Indian patients. Our study population included patients attending cardiology department, PGIMER, Chandigarh. Total 130 patients were included in the study those had an established diagnosis of hypertrophic cardiomyopathy and satisfied the inclusion criteria. Following observations noted in the study. Total 130 patients were followed up, tested and age-based comparison is done in total of 90 patients. Frequency of “MYBPC3” “25 BP” deletion in intron 32 was 4.61 percent. All patients carrying this polymorphism were heterozygous for polymorphism. Family history of Sudden cardiac death was significantly higher in patients carrying polymorphism with P value of 0.015 (83.3 % versus 29.8 % in patients with and without polymorphism respectively). Patients carrying polymorphism had age of onset earlier compared with patients without polymorphism, however difference is not significant {43.33 (+ 14.679) versus 45.34 (+14.18)}. Majority of patients in our study was male {65 (72.22 %)} and were in NYHA class II at presentation {54 (60 %)}. All cause death was higher in patients carrying polymorphism; however, this could not reach statistical significance probably due to low sample size. Similarly, we have noticed, the frequency of syncope and atrial fibrillation were higher in patients carrying polymorphism compared to patients without polymorphism {2 (9.1%) and 1 (6.3 %) versus 4 (5.9%) and 5 (6.4%)}, although not statistically significant.*

**Keywords:** Hypertrophic cardiomyopathy, Genetic mutations, 25 BP deletion, MYBPC3

## 1. Introduction

Hypertrophic cardiomyopathy is a disorder of cardiac muscle consist of myocardial hypertrophy in the absence of abnormal loading conditions. HCM is the most commonly inherited cardiac disease.<sup>9</sup> Hypertrophic cardiomyopathy patients had 1–2% incidence of cardiovascular death per year. However, genetic etiology of HCM remains predominantly unknown.<sup>1</sup> Sarcomeric mutations leading to cardiomyopathies are a major monogenic cause responsible for heart failure. Its prevalence is found to be 1 in 500 in a population of young adults.<sup>2,3</sup>

However, in resource limited countries like India, the molecular etiologies of such cardiomyopathies remain poorly understood. Although usually, transmission occurs in an autosomal dominant manner, recessive mutations have been seen in a few cases.<sup>4,5</sup> Mutations in the “MYBPC3” gene were first identified in 1995<sup>12,13</sup>, and, to date, nearly 200 mutations have been published.<sup>14</sup>

The common causes attributed for death are SCD, HF and thromboembolism.<sup>6</sup> Most of the series showed an overall risk of sudden cardiac death is around  $\leq 1$  percent per year.<sup>7</sup> Mutations of sarcomeric proteins are linked with a high likelihood of sudden cardiac death in HCM patients but the prognostic utility of the genetic testing is limited due to the heterogeneity and variable expression of genetic

abnormalities.<sup>8</sup> Also, it's the most frequent cause of acute and sudden cardiac arrest in young people.<sup>10</sup> Particularly, mutations involving the “MYBPC3” gene are responsible for nearly 40% of all cases.<sup>9,11</sup> The exact functional and pathological consequences of “MYBPC3” mutations are not totally explained because mutant proteins are not typically expressed in myocardial tissue from diseased patients.<sup>15,16</sup>

In the study, we have recruited 130 patients of HCM and tested them for deletion of “25 BP” in “MYBPC3”. We have studied clinical and demographics characteristics of the patients positive for this polymorphism in Indian setting. Also, clinical and demographics characteristics of all HCM patients have also been studied.

## 2. Review of Literature

**Prevalence:** Prevalence in the population, as determined using echocardiography in a variety of ethnic populations around the world, is 0.2 percent (1 out of every 500 adults).

### Diagnosis

The accepted definition, HCM is a disorder characterized by LV hypertrophy which remains unexplained, and associated with non-dilated LV chambers of heart in the absence of other cardiac/systemic disease that may lead to this amount of LV hypertrophy, evident in a patient with the caveat that patients who are positive genotypically, may be

phenotypically negative without evident hypertrophy.<sup>17</sup>  
<sup>18</sup>Standard tool for clinical diagnosis is 2 D echo and now increasingly with CMRI. Genetic testing identifies genetic constitution which is involved in disease and is presently used most effectively in the identification of affected relatives in families with HCM.<sup>19</sup> It's a heterogenous myocardial disease with a diverse clinical manifestations and course, may present in all age groups.<sup>20</sup> Most patients lead a normal life with normal expectancy without disability or the need for major interventions.<sup>21</sup> While some HCM patients, suffers various disease complications that may be serious and may result in disease progression, premature death.<sup>22, 23</sup> SCD is a rare but may be a devastating clinical complication and incidence is around 0.5%-1% per year in patients.<sup>24</sup> The arrhythmias which is commonly linked is spontaneous VF, but asystole, Atrioventricular block and PEA are also seen.<sup>25</sup>

**Mutations in sarcomeric protein genes** — It is inherited as AD mendelian pattern with variability in expression and age related penetrance. Till the date, 15 or more disease causing genes with more than 1500 mutations in genes encoding the proteins of the sarcomere (both thick, thin myofilament) have been found.<sup>26</sup>

**Types of mutations:** The majority of disease-causing mutations in HCM are missense, in which a single normal AA is replaced for another. Other common mutations are frame shift mutations, which may lead to a production of an abnormal short and truncated protein due to the insertion/deletion of one or more nucleic acids.

**Genes** — Most of the loci of genes in familial HCM code for one of the contractile cardiac sarcomere proteins, including Cardiac myosin binding protein C (MYBPC), Cardiac Troponin T, Cardiac Troponin I, Myosin regulatory lightchain (MRLC), Myosin essential light chain (MELC), Cardiac alpha/beta-myosin heavy chain, Cardiac alpha actin, Alpha tropomyosin (TPM), Titin.

**Frequency of identified mutations:** The diagnostic yield of mutation screening for the identification of pathogenic mutations in patients with HCM varies across studies, ranging from 30 to 63 percent.<sup>27</sup> In all studies, mutations involving "MYBPC" are most common, constituting for up to half of the mutations identified. While mutations in the cardiac beta-MYH gene are next in frequency, being present in around 25 to 40 percent of patients.<sup>27</sup> Next in frequency are the mutations involving troponin I, troponin T, and alphaTPM genes which account for only 5 to 10 percent of cases but may present with distinctive phenotypes.<sup>19</sup>

In a study of 84 children diagnosed with isolated unexplained LVH before 15 years of age, mutations in genes associated with HCM in adults were identified in nearly half of sporadic cases and in nearly 2/3 of familial cases.<sup>28</sup> In a study of 79 consecutive patients age 13 or younger who were diagnosed with HCM, "MYBPC3" and MYH7 being the most common (49 and 36 percent of patients respectively).<sup>29</sup>

Jie L et al performed the study in cohort of 179 Chinese patients and found "MYBPC3" is the most predominant

gene mutation in this HCM cohort.<sup>30</sup> Elderly patients may have a different disease from younger patients.<sup>31, 32</sup>

### Aims and Objectives

The aim of the study was to study the frequency of "25 BP" deletion in intron 32 of "MYBPC3" gene along with clinical characteristics of the Hypertrophic Cardiomyopathy patients carrying for this mutation in Indian patients.

### 3. Materials and methods

#### Design:

It was a single centre observational cohort study from PGIMER. This study included the entire patient cohort whose DNA is available with diagnosis of Hypertrophic Cardiomyopathy from a period of April 2003 to February 2018. Diagnosis of Hypertrophic Cardiomyopathy was made in each patient, based on standard diagnostic criteria.

#### Duration:

The study duration comprised of repeat and independent data analysis of patients of HCM cohort from 2003 to 2018 and prospective study through a period from January 2017-February 2018.

#### Follow Up:

All patients, included after the beginning of the study had at least 1 visit after enrolment.

#### Inclusion Criteria:

- 1) Patients of age  $\geq 18$  years
- 2) Disease was defined as a maximum LV thickness  $\geq 15$ mm which is not explained by other abnormal loading conditions or according to the criteria which is published for the disease diagnosis in relatives of patients with unequivocal disease.

#### Exclusion Criteria:

- 1) Patients having known metabolic diseases like Anderson-Fabry disease.
- 2) Patients with syndromes like Noonan syndrome.

Blood samples of the HCM patient was collected for polymorphism analysis. The "25 BP" deletion in intron 32 of "MYBPC3" gene was assessed by RFLP (Restricted fragment length polymorphism). Based on the result, patients was be divided into two cohorts. Symptoms, exercise capacity, morphology and outcome of HCM patients carrying mutation is compared to age, sex match cohort of those without mutation.

#### Data Collection:

A thorough detailed history was taken from the patient and relatives regarding the age of onset, clinical features, family history of sudden cardiac death, history of unexplained syncope. Two-dimensional echocardiography was performed to measure maximal LV thickness, FS, LA diameter, maximal LVOT gradient and other risk factors. Blood samples were collected from all the patients enrolled. The collected samples were stored at -20 degree Celsius in the department of experimental medicine and biotechnology. The samples were processed using FavorPrep Tissue Genomic DNA Extraction Mini Kit.

**Ethical justification**

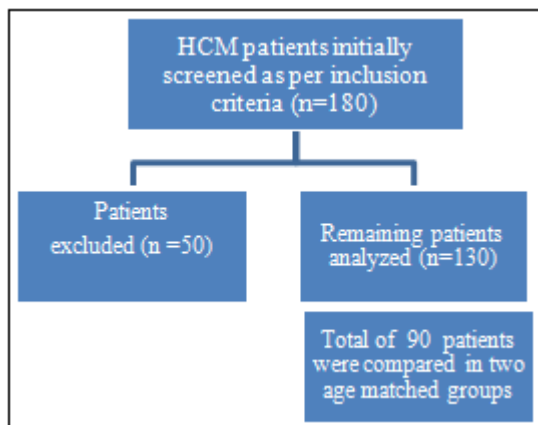
All subjects enrolled after an informed consent of participation into the study. They were duly explained about the tests to be done on them. All the investigations included in the study were done routinely as an evaluation work up in patients. They were assured that all the tests done in this study are a part of the workup for their illness and thus bore no additional cost for them. Each subject can withdraw from the study at any point of time during the course of study, without any prejudices of their right to undergo treatment here in PGI.

**Statistical methods and data analysis procedure**

In this study, we have represented categorical variables as percentages and continuous data which was normally distributed were summarised as mean ± SD and median (inter-quartile range) was used for non-normally distributed data. Student t test or Mann–Whitney U test was applied for the variables which were continuous between groups, and  $\chi^2$  test was used to compare data which was categorical. All tests used were 2-sided and a P value < 0.05 was considered statistically significant. SPSS software version 21 (IBM), and Microsoft Excel 2010 (Microsoft Corporation) was used for statistical analyses.

**4. Observation and Results**

From a period of 2003 to 2018, 180 consecutive patients were included in hypertrophic cardiomyopathy cohort of the department of cardiology, PGIMER, Chandigarh. Patients having at least 1 follow up visit were screened. Out of these 50 patients were excluded due to non-availability of data, lost to follow up or sample loss during processing. The remaining 130 patients were included in the study, tested and analysed. Total of 90 age matched patients in two groups were compared.



**Figure 1:** Study design

**Table 1:** Baseline characteristics of patients

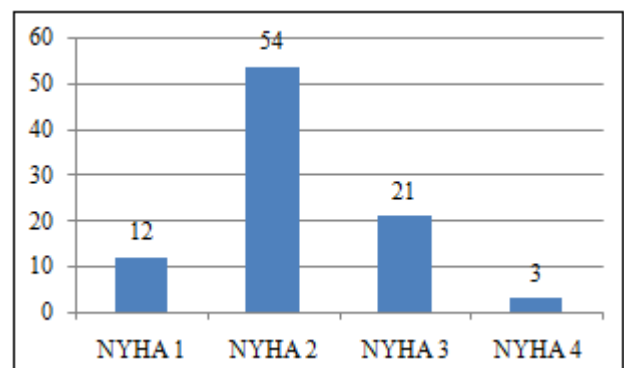
| Parameters | Total number (percentage/Mean + SD) |
|------------|-------------------------------------|
| Age        | 45.34 (+14.18)                      |
| Male       | 65 (72.22 %)                        |
| Female     | 25 (27.77 %)                        |
| NYHA1      | 12 (13.33 %)                        |
| NYHA2      | 54 (60 %)                           |
| NYHA3      | 21 (23.33 %)                        |
| NYHA4      | 3 (3.33 %)                          |
| F/H/O SCD  | 29 (32.22 %)                        |

|                              |                 |
|------------------------------|-----------------|
| NSVT                         | 27 (30 %)       |
| Syncope                      | 22 (24.44 %)    |
| Abnormal BP response         | 12 (13.33%)     |
| LV thickness (mm)            | 23.13 (+ 5.66)  |
| LV thickness more than 30 mm | 9 (10 %)        |
| LA diameter                  | 41.46 (+ 7.95)  |
| LVOT gradient (mmhg)         | 22.57 (+ 27.80) |
| LVED (mm)                    | 38.37 (+ 7.10)  |
| FS%                          | 46.19 (+ 10.79) |
| Myomectomy                   | 2 (2.22 %)      |
| Alcohol ablation             | 13 (14.44 %)    |
| AF                           | 12 (12.6 %)     |
| Amiodarone                   | 12 (13.3 %)     |
| ICD                          | 4 (4.4 %)       |
| All cause death              | 12 (13.3 %)     |

Finally, 130 HCM patients analysed, 90 age matched patients were divided, compared and their baseline characteristics are listed in table no 1. The mean age of presentation was 45.34 ± 14.18 years and male patients were about 2.6 times more frequent than female patients (72.22% Vs 27.77%). Most of the patients presented in NYHA II and NYHA III status 60% and 23.33% respectively. Among the patients about 32.22% patients had positive family history for SCD and about 24.44% patients had experienced at least 1 episode of syncope. On Holter monitoring 30 % patients showed runs of non-sustained ventricular tachycardia. Around 13.33% patients had abnormal BP response on treadmill test. All patients underwent 2D echocardiography and the mean LA diameter was about 41.46 ± 7.95 mm. Mean left ventricular hypertrophy was 23.13 ± 5.66 mm and 10 % patients had left ventricular thickness ≥ 30 mm. Baseline left ventricular outflow gradient was around 22.57 ± 27.80 mm Hg. Total 14.44% patients had undergone alcohol septal ablation and 2.22% patients had surgical Myomectomy. About 13.3% patients had documented atrial fibrillation. For primary prevention of SCD 4.4 % patients got ICD implanted. There were 12 deaths in our study population over a period of follow up.

**Distribution of patients according NYHA status**

As we can see in following figure, majority of patients with HCM belong to NYHA class II (54 out of 90; 60 %), followed by NYHA III and least being in NYHA IV. The distribution of all patients according to NYHA class at presentation is depicted in Figure 2.



**Figure 2:** NYHA status of HCM patients at the time of presentation

**Gender distribution**

As shown diagrammatically in Figure 3, there was predominance of male population in our study. Out of total 90 patients, we had 65 male patients and rests were females.

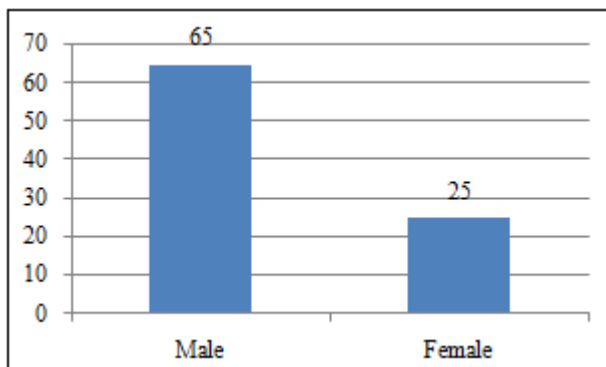


Figure 3: Gender distribution of study population

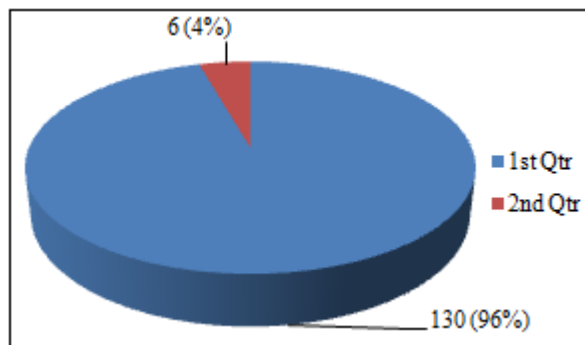


Figure 4: Frequency of "MYBPC3" in study population

**Characteristics of patients with polymorphism**

Table 2: Baseline characteristics of patients carrying polymorphism

| Parameters           | Total number (percentage/ Mean + SD) |
|----------------------|--------------------------------------|
| Number               | 6 (4.61%)                            |
| Age                  | 43.33 (+ 14.679)                     |
| Male                 | 4 (66.66 %)                          |
| Female               | 2 (33.33 %)                          |
| NYHA1                | 1 (16.6 %)                           |
| NYHA2                | 5 (83.33 %)                          |
| NYHA3                | 0                                    |
| NYHA4                | 0                                    |
| F/H/O SCD            | 4 (66.66 %)                          |
| NSVT                 | 2 (33.33 %)                          |
| Syncope              | 2 (33.33 %)                          |
| LV thickness (mm)    | 23.13 (+ 5.66)                       |
| LA diameter          | 41.46 (+ 7.95)                       |
| LVOT gradient (mmhg) | 22.57 (+ 27.80)                      |
| LVed (mm)            | 38.37 (+ 7.10)                       |
| FS%                  | 46.19 (+ 10.79)                      |
| Alcohol ablation     | 0                                    |
| AF                   | 1 (16.6 %)                           |
| All cause death      | 1 (16.6 %)                           |

**Frequency of "MYBPC3"**

The frequency of "MYBPC3" in current study is 4.61 percent. Total of 6 patients had polymorphism (heterozygous) out of 130 patients screened. So, the frequency found in our study in Indian patients correlate with frequency of polymorphism noted in other studies in Asian population.

**Age at diagnosis**

Mean age of patients at presentation was earlier in patients carrying polymorphism (43.33+14.67 versus 45.49+ 14.23) with P value of 0.740.

Table 3: Age (at diagnosis) distribution in two groups

|                  | Group Statistics |    |       |                |         |
|------------------|------------------|----|-------|----------------|---------|
|                  | "MYBPC3"         | N  | Mean  | Std. Deviation | P value |
| Age at diagnosis | 1                | 6  | 43.33 | 14.67          | 0.740   |
|                  | 2                | 84 | 45.49 | 14.231         |         |

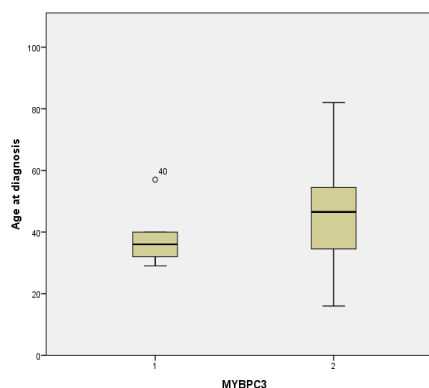


Figure 5: Box plot showing age distribution of patients in two groups

**Gender distribution**

The distribution of gender was similar in both groups, that is with polymorphism and without polymorphism (66.66% versus 67.77%) with P value of 0.66.

Table 4: Gender distribution in two groups

| "MYBPC3" * Gender |   |     |    |       | P value |
|-------------------|---|-----|----|-------|---------|
| Count             |   |     |    |       |         |
|                   |   | Sex |    | Total |         |
|                   |   | F   | M  |       |         |
| "MYBPC3"          | 1 | 2   | 4  | 6     | 0.66    |
|                   | 2 | 23  | 61 | 84    |         |
| Total             |   | 25  | 65 | 90    |         |

**Comparison between FHO SCD and presence of polymorphism**

In our study, we have found that majority of patients with polymorphism has positive family history of SCD, while minority had in patients without polymorphism. The difference was significant with P value of 0.015.

**Table 5:** Comparison between FHO SCD and presence of “MYBPC3” deletion

| “MYBPC3” * FHOSCD |                   |                   |       |        |        | P value |
|-------------------|-------------------|-------------------|-------|--------|--------|---------|
|                   |                   | fhoSCD            |       |        | Total  |         |
|                   |                   | 1                 | 2     |        |        |         |
| “MYBPC3”          | 1                 | Count             | 5     | 1      | 6      | 0.015   |
|                   |                   | % within “MYBPC3” | 83.3% | 16.7%  | 100.0% |         |
|                   | 2                 | Count             | 25    | 59     | 84     |         |
|                   |                   | % within “MYBPC3” | 29.8% | 70.2%  | 100.0% |         |
| Total             | Count             | 30                | 60    | 90     |        |         |
|                   | % within “MYBPC3” | 33.3%             | 66.7% | 100.0% |        |         |

**Comparison between All cause death and presence of “MYBPC3” deletion**

In our study, we have found no significant difference in mortality in between the patients carrying polymorphism and those without polymorphism. There was a single death

in patients carrying polymorphism (1 out of 6) and there 11 deaths in patients without polymorphism (11 out of 84). Although there was no significant difference between the two groups (0.587), but there was trend towards higher mortality in patients carrying polymorphism.

**Table 6:** Distribution of All cause death in two groups

| All Cause Death * “MYBPC3” polymorphism |                          |                          |       |        |        | P value                        |
|---|--------------------------|--------------------------|-------|--------|--------|--------------------------------|
|   |                          | “MYBPC3”                 |       | Total  |        |                                |
|   |                          | 1                        | 2     |        |        |                                |
| All cause death                         | 1                        | Count                    | 1     | 11     | 12     | 0.587<br>(Fisher's Exact Test) |
|   |                          | % within all cause death | 8.3%  | 91.7%  | 100.0% |                                |
|   | 2                        | Count                    | 5     | 73     | 78     |                                |
|   |                          | % within all cause death | 6.4%  | 93.6%  | 100.0% |                                |
| Total                                   | Count                    | 6                        | 84    | 90     |        |                                |
|   | % within all cause death | 6.7%                     | 93.3% | 100.0% |        |                                |

**Five-year risk of sudden cardiac death**

Five year risk of sudden cardiac death was non-significantly higher in patient group carrying polymorphism than those without this polymorphism with P value of 0.637.

**Table 7:** Comparison of Five year risk SCD between two groups

| Group Statistics |          |    |               |                |         |
|------------------|----------|----|---------------|----------------|---------|
|                  | “MYBPC3” | N  | Mean          | Std. Deviation | P value |
| Five YrRiskSCD   | 1        | 5  | 5.21949131160 | 2.13           | 0.637   |
|                  | 2        | 83 | 4.51975393884 | 3.24           |         |

**Comparison between syncope and presence of “MYBPC3” deletion**

We have found that 2 of 6 (9.1%) patients carrying polymorphism had syncope while 20 of 84 (5.9%) patients without polymorphism had the syncope. So, patients with polymorphism had a higher rate of syncope, however this was not significant with P value of 0.632.

**Table 8:** Comparison of syncope between two groups

| H/O Syncope * “MYBPC3” |                    |                    |       |        |        | P value                      |
|------------------------|--------------------|--------------------|-------|--------|--------|------------------------------|
|                        |                    | “MYBPC3”           |       | Total  |        |                              |
|                        |                    | 1                  | 2     |        |        |                              |
| hosyncope              | 1                  | Count              | 2     | 20     | 22     | 0.632<br>Fisher's Exact Test |
|                        |                    | % within hosyncope | 9.1%  | 90.9%  | 100.0% |                              |
|                        | 2                  | Count              | 4     | 64     | 68     |                              |
|                        |                    | % within hosyncope | 5.9%  | 94.1%  | 100.0% |                              |
| Total                  | Count              | 6                  | 84    | 90     |        |                              |
|                        | % within hosyncope | 6.7%               | 93.3% | 100.0% |        |                              |

**Comparison between AF and presence of “MYBPC3” polymorphism**

In our study, we have found 1 out of 6 patients with polymorphism has atrial fibrillation, while 11 out of 84 had in patients without mutation. However, the difference is not significant with P value of 0.587.

**Table 9:** Comparison of atrial fibrillation between two groups

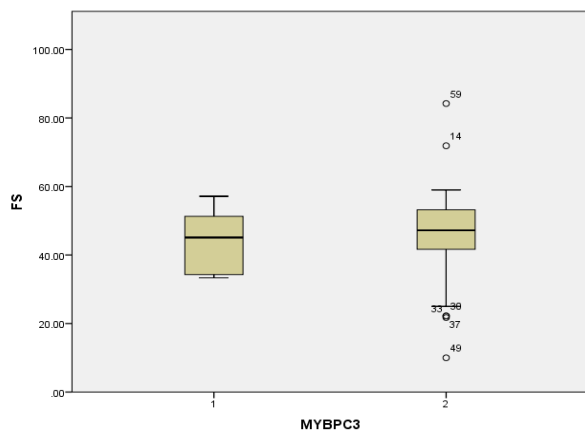
| AF * “MYBPC3” deletion |             |             |       |        |        | P value                        |
|------------------------|-------------|-------------|-------|--------|--------|--------------------------------|
|                        |             | “MYBPC3”    |       | Total  |        |                                |
|                        |             | 1           | 2     |        |        |                                |
| AF                     | 1           | Count       | 1     | 11     | 12     | 0.587<br>(Fisher's Exact Test) |
|                        |             | % within AF | 8.3%  | 91.7%  | 100.0% |                                |
|                        | 2           | Count       | 5     | 73     | 78     |                                |
|                        |             | % within AF | 6.4%  | 93.6%  | 100.0% |                                |
| Total                  | Count       | 6           | 84    | 90     |        |                                |
|                        | % within AF | 6.7%        | 93.3% | 100.0% |        |                                |

**Fractional shortening**

Fractional shortening was non-significantly lower in patients carrying polymorphism than patients without polymorphism, with P value of 0.647.

**Table 10:** Comparison of Fractional shortening between two groups

| Group Statistics |          |    |         |                |         |
|------------------|----------|----|---------|----------------|---------|
|                  | “MYBPC3” | N  | Mean    | Std. Deviation | P value |
| FS               | 1        | 6  | 44.3805 | 9.41348        | 0.647   |
|                  | 2        | 83 | 46.3212 | 10.92716       |         |



**Figure 6:** Box plot showing distribution of FS between two groups

### Death in polymorphism group

There was a single death in mutation category which was due to non-cardiac cause. The single death in polymorphism group was due to respiratory failure.

### Other parameters

No significant difference was found in other parameters between two groups like NSVT, abnormal BP response, alcohol ablation, SCD and ICD. Similarly, no significant differences in various parameters like NYHA status, LV thickness, LA diameter, LVOT gradient between two groups.

## 5. Discussion

In the present study, we examined the prevalence and spectrum of mutations in “MYBPC3” which is “25 BP” deletion in intron 32.

The aim of the thesis is to study the frequency “25 BP” deletion in intron 32 of “MYBPC3” gene along with clinical characteristics of the Hypertrophic Cardiomyopathy patients carrying for this mutation in Indian patients. We have tested the samples of 130 patients for the mutation. Out of these 130 patients, 6 patients have polymorphism positive. The clinical and laboratory data were studied for these patients. Subsequently comparison was done between the patients with positive mutation and patients of similar age group.

### Frequency of polymorphism

In our study, out of 130 patients tested for polymorphism, 6 patients were positive for polymorphism. So frequency of “MYBPC3” “25 BP” deletion was 4.61 percent. A study by Perunduraj S Dhandapany et al, found relatively high prevalence (~4%) in populations of Indian subcontinental ancestry. So, result of our study correlates with this and other previous study.<sup>3</sup>

### Polymorphisms in “MYBPC3” gene

We observed a very low prevalence of polymorphism in “MYBPC3” gene (4.25%) in North Indians as compared to relatively high frequency (11.57%) in Japanese, Caucasians and Europeans (15-25%).<sup>3,4</sup> This low frequency may be attributed to the fact that we did not screen all the exons as done in these studies. However, a similar study involving the screening of hotspots of polymorphisms in “MYBPC3” in

South Indian population has also reported a relatively high (11.57%) prevalence of polymorphisms in HCM patients.<sup>5</sup> But as we know, North Indian population (Indo-Europeans) is very different from South Indian population (Dravidians), so difference in prevalence of genetic variations can be attributed to ethnic and genetic differences between these populations. The earlier reported polymorphism was identified in 6 Proband in HCM. The symptoms of these patients were variable (mild to severe hypertrophy) with early onset of the disease.

### Demographics characteristic

Mean age of patients in our study is 45.34 (+14.18), while the mean of patients with polymorphism is 43.33 (+14.67). However, this difference was not significant with P value of 0.740, probably due to smaller sample size. So study with larger population size may be able to show significant difference. But, there is trend towards the early presentation of patients carrying this polymorphism suggesting its role in pathogenesis. So larger study will help to confirm this finding.

Also, majority of patients in our study was male {65 (72.22 %) in control group and 4 (66.66 %) in patients with polymorphism}. So there was male predominance in our study. The gender distribution was similar between the two groups and difference was not significant. This is probably related to symptoms awareness and gender inequality in our country leading to larger number of males presenting to tertiary care hospital. Population based studies have shown, equal gender distribution for both genders.

### NYHA status

Majority of population was in NYHA class 2 status at presentation {54 (60 %) in control group versus 5 (83.33 %) in mutation positive group}. On analysis, we did not find significant difference between two groups in terms of dyspnoea class at examination. Also, various previous studies shown that, majority of HCM patients fall in NYHA class I and II.

### Comparison between FHO SCD and presence of “MYBPC3” deletion

We have found that, significantly more number of patients had positive family history of SCD in patients with polymorphism, with P value of 0.015. This suggests the possibility of deleterious effect of polymorphism on clinical manifestation. However, this is the indirect evidence of disease modification, rather than direct evidence of mortality in patients with HCM. We were not able to demonstrate the significant difference in mortality in our patients probably due to less number of events due to small sample size. So, this demand the larger study to be conducted with larger sample size.

### All cause death

In this study, we have found no significant difference between the two groups in terms of mortality. Partly, this may be due to less number of events in patients carrying polymorphism which is probably due to smaller sample size. There was a single death in patients carrying polymorphism and it was attributable to non-cardiac cause. So study needs

to be carried out with larger sample size to get significant difference.

### Other parameters

We have also compared other parameters like Five year risk of sudden cardiac death, prevalence of syncope, prevalence of atrial fibrillation. We have noticed in our study, the five year risk of sudden cardiac death was lower in patients carrying polymorphism, but this could not reach statistical significance with P value of 0.637.

We have found similar results for prevalence of syncope and atrial fibrillation with P value being 0.632 and 0.587 respectively. Probable reason for this is inadequate number of events due to smaller sample size. So, it warrants evaluation in larger study.

## 6. Limitations

A single center study, results need to be verified at multiple centers in different populations. Small cohort of patients and small number of patients with polymorphism, needs larger study to ascertain the results. Selection and reference bias as the study was conducted in a tertiary center of north India and therefore the patient population might differ from general hypertrophic cardiomyopathy population. Majority of study population was male population creating gender bias.

## 7. Conclusion

So according to findings it was concluded from our study that frequency of “25 BP” deletion” in intron 32 of “MYBPC3” in HCM patients in North India is 4.61 percent. Significantly higher number of patients with polymorphism had positive family history of sudden cardiac death. Majority of study population was male. Age at diagnosis was lower in patients with polymorphism, but could not reach statistical significance. Five-year risk of SCD, risk of syncope and AF was higher in patients with polymorphism, but could not reach statistical significance probably due to less sample size.

## References

- [1] Reddy, K. S., Shah, B., Varghese, C. & Ramadoss, A. Responding to the threat of chronic diseases in India. *Lancet* 366, 1744–1749 (2005).
- [2] Marian AJ, Mares A, Jr, Kelly DP, et al. Sudden cardiac death in hypertrophic cardiomyopathy. Variability in phenotypic expression of beta-myosin heavy chain mutations. *Eur Heart J*.1995; 16: 368–76.
- [3] McKenna WJ, Behr ER. Hypertrophic cardiomyopathy: Management, risk stratification, and prevention of sudden death. *Heart*.2002; 87: 169–76.
- [4] Jääskeläinen P, Soranta M, Miettinen R, et al. The cardiac beta-myosin heavy chain gene is not the predominant gene for hypertrophic cardiomyopathy in the Finnish population. *J Am Coll Cardiol*.1998; 32: 1709–16. [PubMed]
- [5] Nishi H, Kimura A, Harada H, et al. Possible gene dose effect of a mutant cardiac beta-myosin heavy chain gene on the clinical expression of familial hypertrophic cardiomyopathy. *Biochem Biophys Res Commun*.1994; 200: 549–56. [PubMed]
- [6] Elliott PM, Gimeno JR, Thaman R, Shah J, Ward D, Dickie S, Tome Esteban MT, McKenna WJ. Historical trends in reported survival rates in patients with hypertrophic cardiomyopathy. *Heart* 2006; 92: 785–791.
- [7] Maron BJ, Olivotto I, Spirito P, Casey SA, Bellone P, Gohman TE et al. Epidemiology of hypertrophic cardiomyopathy-related death: revisited in a large non-referral-based patient population. *Circulation* 2000; 102: 858–64.
- [8] Watkins H, McKenna WJ, Thierfelder L, et al. Mutations in the genes for cardiac troponin T and alpha-tropomyosin in hypertrophic cardiomyopathy. *N Engl J Med* 1995; 332: 1058–64.
- [9] Richard, P., Charron, P., Carrier, L., Ledeuil, C., Cheav, T., Pichereau, C., Benaiche, A., Isnard, R., Dubourg, O., Burban, M., Gueffet, J. P., Millaire, A., Desnos, M., Schwartz, K., Hainque, B., and Komajda, M. (2003) Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation* 107, 2227–2232.
- [10] Maron, B. J., Doerer, J. J., Haas, T. S., Tierney, D. M., and Mueller, F. O. (2009) Sudden deaths in young competitive athletes: analysis of 1866 deaths in the United States, 1980–2006. *Circulation* 119, 1085–1092.
- [11] Alcalai, R., Planer, D., Culhaoglu, A., Osman, A., Pollak, A., and Lotan, C. (2007) Acute coronary syndrome vs nonspecific troponin elevation: clinical predictors and survival analysis. *Arch. Intern. Med.* 167, 276–281.
- [12] Bonne, G., Carrier, L., Bercovici, J., Cruaud, C., Richard, P., Hainque, B., Gautel, M., Labeit, S., James, M., Beckmann, J., Weissenbach, J., Vosberg, H. P., Fiszman, M., Komajda, M., and Schwartz, K. (1995) Cardiac myosin binding protein-C gene splice acceptor site mutation is associated with familial hypertrophic cardiomyopathy. *Nat Genet.* 11, 438–440.
- [13] Watkins, H., Conner, D., Thierfelder, L., Jarcho, J. A., MacRae, C., McKenna, W. J., Maron, B. J., Seidman, J. G., and Seidman, C. E. (1995) Mutations in the cardiac myosin binding protein-C gene on chromosome 11 cause familial hypertrophic cardiomyopathy. *Nat. Genet.* 11, 434–437.
- [14] Harris, S. P., Lyons, R. G., and Bezold, K. L. (2011) In the thick of it: HCM-causing mutations in myosin binding proteins of the thick filament. *Circ. Res.* 108, 751–764.
- [15] Rottbauer, W., Gautel, M., Zehelein, J., Labeit, S., Franz, W. M., Fischer, C., Vollrath, B., Mall, G., Dietz, R., Ku'bler, W., and Katus, H. A. (1997) Novel splice donor site mutation in the cardiac myosin binding protein-C gene in familial hypertrophic cardiomyopathy. Characterization of cardiac transcript and protein. *J. Clin. Invest.* 100, 475–482.
- [16] VanDijk, S. J., Dooijes, D., dos Remedios, C., Michels, M., Lamers, J. M., Winegrad, S., Schlossarek, S., Carrier, L., tenCate, F. J., Stienen, G. J., and van der Velden, J. (2009) Cardiac myosin binding protein-C

- mutations and hypertrophic cardiomyopathy: haploinsufficiency, deranged phosphorylation, and cardiomyocyte dysfunction. *Circulation* 119, 1473–1483.
- [17] Maron BJ, Seidman CE, Ackerman MJ, et al. How should hypertrophic cardiomyopathy be classified? What's in a name? Dilemmas in nomenclature characterizing hypertrophic cardiomyopathy and left ventricular hypertrophy. *Circ Cardiovasc Genet* 2009; 2: 81–85.
- [18] Maron BJ, Yeates L, Semsarian C. Clinical challenges of genotype positive (+)-phenotype negative (-) family members in hypertrophic cardiomyopathy. *Am J Cardiol* 2011; 107: 604–8.
- [19] Gersh BJ, Maron BJ, Bonow RO, Dearani JA, Fifer MA, Link MS et al; American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation* 2011; 124: 2761–96.
- [20] Maron BJ. Hypertrophic cardiomyopathy: a systematic review. *JAMA*.2002; 287: 1308–20.
- [21] Cannan CR, Reeder GS, Bailey KR, et al. Natural history of hypertrophic cardiomyopathy: a population-based study, 1976 through 1990. *Circulation*.1995; 92: 2488–95.
- [22] Watkins H, McKenna WJ, Thierfelder L, et al. Mutations in the genes for cardiac troponin T and alpha-tropomyosin in hypertrophic cardiomyopathy. *N Engl J Med* 1995; 332: 1058–64.
- [23] Maron BJ, McKenna WJ, Danielson GK, Kappenberger LJ, Kuhn HJ, Seidman CE et al. American College of Cardiology/European Society of Cardiology clinical expert consensus document on hypertrophic cardiomyopathy. A report of the American College of Cardiology Foundation Task Force on clinical expert consensus documents and the European Society of Cardiology Committee for practice guidelines. *J Am Coll Cardiol* 2003; 42: 1687–713.
- [24] Elliott PM, Anastakis A, Borger MA, Borggrefe M, Cecchi F, Charron P et al.2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy: the Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). *Eur Heart J*.2014; 35: 2733–79.
- [25] Barriales-Villa R, Centurion-Inda R, Fernandez-Fernandez X, Ortiz MF, Perez-Alvarez L, Rodriguez G I et al. Severe cardiac conduction disturbances and pacemaker implantation in patients with hypertrophic cardiomyopathy. *Rev Esp Cardiol* 2010; 63: 985–88.
- [26] Ingles J, Burns C, Barratt A, Semsarian C. Application of Genetic Testing in Hypertrophic Cardiomyopathy for Preclinical Disease Detection. *Circ Cardiovasc Genet* 2015; 8: 852.
- [27] Richard P, Charron P, Carrier L, et al. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation* 2003; 107: 2227.
- [28] Morita H, Rehm HL, Menesses A, et al. Shared genetic causes of cardiac hypertrophy in children and adults. *N Engl J Med* 2008; 358: 1899.
- [29] Kaski JP, Syrris P, Esteban MT, et al. Prevalence of sarcomere protein gene mutations in preadolescent children with hypertrophic cardiomyopathy. *Circ Cardiovasc Genet* 2009; 2: 436.
- [30] Jie L et al.2015 Aug; 43 (8): 682-9. Mutation and clinical relevance in a large cohort of unrelated Chinese patients with hypertrophic cardiomyopathy.2015 Aug; 43 (8): 682-9.
- [31] Lever HM, Karam RF, Currie PJ, Healy BP. Hypertrophic cardiomyopathy in the elderly. Distinctions from the young based on cardiac shape. *Circulation* 1989; 79: 580.
- [32] Lewis JF, Maron BJ. Elderly patients with hypertrophic cardiomyopathy: a subset with distinctive left ventricular morphology and progressive clinical course late in life. *J Am Coll Cardiol* 1989; 13: 36.
- [33] Dhandapany, P. S., Sadayappan, S., Xue, Y., Powell, G. T., Rani, D. S., Nallari, P., Rai, T. S., Khullar, M., Soares, P., Bahl, A., Tharkan, J. M. Vaideeswar, P., Rathinavel, A., Narasimhan, C., Ayapati, D. R., Ayub, Q., Mehdi, S. Q., Oppenheimer, S., Richards, M. B., Price, A. L., Patterson, N., Reich, D., Singh, L., TylerSmith, C., and Thangaraj, K. (2009) A common “MYBPC3” (cardiac myosin binding protein-C) variant associated with cardiomyopathies in SouthAsia. *Nat. Genet.*41, 187–191.
- [34] Anan R, Niimura H, Takenaka T, Hamasaki S, Tei C. Mutations in the Genes for Sarcomeric Proteins in Japanese Patients With Onset Sporadic Hypertrophic Cardiomyopathy After Age 40 Years. *Am J Cardiol*.2007 Jun 15; 99 (12): 1750–4.