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Association of MYBPC3 Gene Polymorphism with Cardiomyopathy Susceptibility in the Jammu Region

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ABSTRACT

Cardiomyopathy is a multifactorial disorder caused by hereditary and environmental factors. The MYBPC3 gene produces cardiac myosin binding protein C (cMyBP-C), which is found in heart (cardiac) muscle cells. This is linked to thick filaments and plays a structural and regulatory role in the contraction of cardiomyocytes. The *MYBPC3Δ25bp* deletion has been linked to an increased vulnerability to cardiomyopathy. The purpose of this study was to investigate the relationship between the MYBC3 gene polymorphism and the risk of cardiomyopathy in a case-control study from the Jammu region of J&K, UT.

Method: A total of 200 subjects were enrolled for the present study, out of which 100 were clinically diagnosed cases of cardiomyopathy and 100 were healthy age matched controls. Genotyping of cases and controls for *MYBPC3* polymorphism was done by using Polymerase Chain Reaction (PCR). A statistical analysis was done to ascertain the association of the above said polymorphism with the risk of cardiomyopathy in the population of Jammu region of J&K, UT.

Results: The present study revealed the significant association of *MYBPC3Δ25bp* (rs36212066) gene polymorphism with an increased risk of cardiomyopathy (p=0.03) in our population. The frequency of deletion allele (risk) was found to be higher in cases (10%) than in controls (2%). Further the study indicated that the *MYBPC3 Δ25bp* allele adds risk for the development of cardiomyopathy in our cases compared to controls [OR (95%CI)- 5.44 (1.16 to 25.52)].

Conclusion: The study found the significant association of *MYBPC3 Δ25bp* polymorphism with Cardiomyopathy in population of Jammu region.

Keywords: *polymorphism, Jammu, Cardiomyopathy, MYBPC3*

1. INTRODUCTION

Cardiomyopathy is a condition that affects the heart muscle. It is a diverse set of cardiac illnesses characterized by mechanical and/or electrical dysfunction, which frequently includes aberrant ventricular hypertrophy or dilatation (Rai et al., 2008). They can be caused by a variety of sources, the most prevalent of which are hereditary. It is divided into various subgroups according to its structural features: restrictive (RCM), dilated (DCM), hypertrophic (HCM), left ventricular noncompaction (LVNC), and arrhythmogenic right ventricular cardiomyopathy (ARVD/C) (Gerull et al., 2019).The most common types of cardiomyopathies are DCM and HCM, which typically impact the size of the heart chambers, the thickness of the heart wall, and eventually the pumping efficiency (Richard et al., 2006).Ventricular chamber enlargement and systolic dysfunction with normal LV wall thickness are characteristics of dilated types of cardiomyopathies; this condition is typically diagnosed by

two-dimensional echocardiography. DCM causes conduction system anomalies, thrombo-embolism, ventricular and supraventricular arrhythmias, a reduction in LV contractile performance, progressive heart failure, and sudden or heart failure-related mortality (Rai et al., 2008). *MYBPC3* mutations, which affect the gene that codes for myosin-binding protein C, are linked to dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) (Xu et al., 2010; Watkins et al., 2011).The structure and sequencing of the human MYBPC3 gene were initially described by Carrier et al. (1997). This is a crucial structural protein of the heart muscle, cardiac myosin-binding protein C (cMyBP-C), is encoded by this gene. Actin, myosin, and titin are all interacting with cMyBP-C, which is essential for preserving sarcomere integrity (Shaffer et al., 2009; Flavigny et al., 2010). Over 60% of all known mutations in *MYBPC3* are truncating variations, which include nonsense mutations, insertions or deletions, and changes in the branch point or splicing. Important myosin- and/or titin-binding sites are therefore absent from cMyBP-C, which is truncated at the COOH terminus (Behrens-Gawlik et al., 2014). The MYBPC3 mutation is characterized by a 25-bp deletion in the intron upstream of exon 33 (Fig. 1). According to transcriptional research, this loss causes exon 33 to be skipped (Dhandapany et al., 2009). Moreover, late-onset cardiomyopathy could be influenced by the buildup of the modified protein (Niimura, 1998; Kubo et al., 2005).

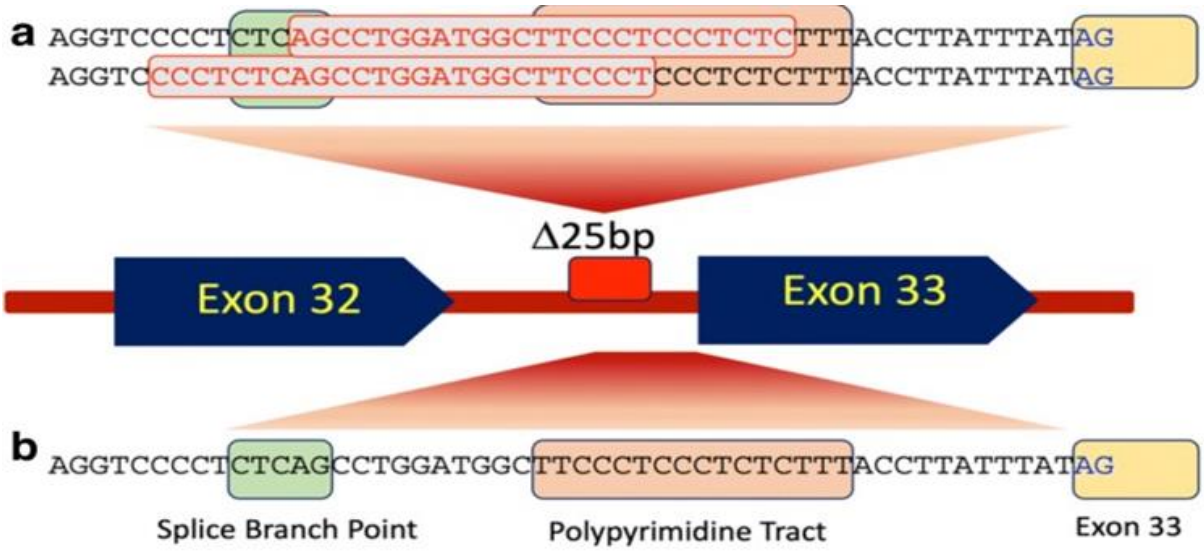

Figure 1: Genotype of MYBPC3Δ25bp in intron 32 of the MYBPC3 gene. (a) Two options of 25-bp deletion are indicated with the same outcome. (b) The location of the splice branch point and polypyrimidine track at the junction of intron 32/exon 33 splicing [Reference: Arif, M., Nabavizadeh, P., Song, T et.al, 2020].

2. MATERIAL AND METHODS

Inclusion criteria: Echocardiography (ECG) technique would be used to diagnose the suspected individuals, where if subjects (both child and adult) have left ventricular enlargement greater than or equal to 13 mm or >2 standard deviations corrected for body surface area, the patient shall be diagnosed with HCM. LVEF (left ventricular ejection fraction) measured by echocardiography is 40% (Rai and Ahmad et. al., 2009).

Exclusion criteria: Patients with an autoimmune condition, malignancy, coronary artery disease (CAD), and advanced chronic renal failure (CRF) or any other systemic or cardiac condition that could cause wall thickening of this magnitude shall not be included in the study (Rai and Ahmad et. al., 2009).

The present study was approved by the Government Medical College Jammu, with ethical clearance granted under reference number IEC/GMC/2022/1143, ensuring compliance with established ethical guidelines. Patients diagnosed with cardiomyopathy were selected by the clinician. Comprehensive clinical evaluations, including electrocardiography (ECG) and other diagnostic procedures, were conducted. For the control group, hospital-based sampling was utilized, selecting participants from the hospital's general outpatient department. These individuals underwent screening to confirm the absence of cardiomyopathy, other cardiac conditions, or hereditary diseases. Prior to participation, each patient was thoroughly informed in their local language and Hindi, about the study's objectives, procedures, and purpose. Written consent was obtained from all participants, affirming their voluntary involvement in the study. Participants' blood samples were collected and transferred to 5 mL EDTA vials to stop clotting. Following their safe transportation, these vials were taken to the Institute of Human Genetics in Jammu. To preserve the sample for later processing, they were promptly put in a freezer set at -20°C upon arrival. During patients' recruitment and data gathering privacy, and confidentiality of participants information, strict ethical guidelines were followed at every stage.

DNA isolation

DNA was extracted from blood samples obtained from both patients and controls using the conventional phenol-chloroform-isoamyl alcohol procedure. After extraction, 1 µl of 1× DNA loading dye containing glycerol, EDTA, and bromophenol blue was added to each sample, and the samples were loaded onto a gel for examination to evaluate the quality of the DNA. To guarantee precise quantification for subsequent tests, the concentration of DNA was assessed using spectrophotometry.

In the current study, we used the UCSC Genome Browser to perform in silico PCR using predesigned primers (https://genome.ucsc.edu/). Forward primer 5'-GTTTCCAGCCTTGGGCATAGTC-3'and Reverse primer 3'-GAGGACAACGGAGCAAAGCCC-5'the genotypes of the variants under study were amplified. The target DNA was amplified by optimizing the PCR reaction mixture, which included Nuclease-free water (3.5µl), forward primer (0.5 µl), reverse primer (0.5 µl), genomic DNA (2 µl), and readymade master mix (6.5µl, Genes2me) total volume (13µl).An initial denaturation at 95°C for 5 minutes was followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 63°C for 30 seconds, and extension at 72°C for 30seconds. A final extension at 72°C for 5 minutes was carried out for the guarantee that all DNA fragments were fully extended. For effective and precise amplification of the target region for further research, these parameters were meticulously adjusted. The PCR product was electrophoresed on a 3% agarose gel and then seen using the Chemi Doc XRS+ Gel Imaging System (Bio-Rad) (Figure1).

BMI calculation

The BMI value was determined by entering the person's height (in centimeters) and weight (in kilograms) into an online BMI calculator. The age of the individual was also factored into the computation.

Statistical Analysis

The relationship between cardiomyopathy and genetic variation was investigated by using statistical analysis. A descriptive statistic in Microsoft Excel, the study population demographics were compiled, and genotype and allele frequencies were ascertained. In SPSS version 25, a t-test was used to compare the means of the case and control groups. To evaluate the strength of relationship across several genetic models, MedCalc was used to compute odds ratios (OR) and 95% confidence intervals (CI). A p-value of less than 0.05 was considered statistically significant. Direct counting was used to assess allele frequencies and allelic frequencies between cases and controls were compared by using Fisher's exact test.

3. RESULTS

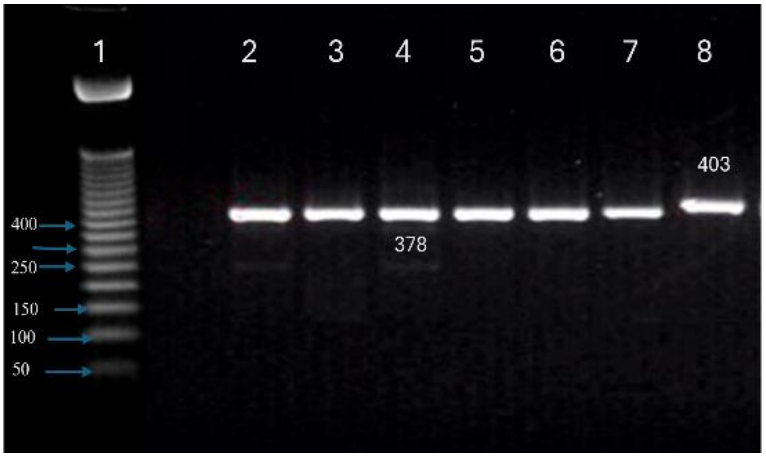

Figure 2: Well 1 represents the 50 bp ladder. Wells 2-7and 12-15 represent homozygous mutant genotype (DD) (25bp deletion allele) 378bp. Well 8 represents homozygous wild genotype (WW) 403bp.

Genetic Association

For the *MYBPC3* variant, In the case group, 90 people have the (WW) wild genotype and 20 have the (DD) mutant genotype allele, according to the genotypic frequencies. 98 carry WW and 2 carry DD in the control group. The control group displayed a higher frequency of homozygous wild-type genotypes (98% vs. 90%) and a higher wild-type allele frequency (0.90 vs. 0.91) as compared to the case group. And in case risk allele is higher in frequency as compared to controls. Even though both groups are predominately W carriers, the D allele is more frequent in cases than control group (0.10 vs 0.02). A statistically significant difference in the genotype/allele distribution between cases and controls is shown by the[OR = 5.44; CI: 1.16 to 25.52; $p<0.03$] (usually, $p < 0.05$ is considered significant)(Table 1).People with the D allele are around 5.4 times more likely to be in the case group (i.e., have the disease) than people without it. Since the whole range is above 1, the result is considered statistically significant, and the D allele may be positively related with illness risk, according to the 95%

Confidence Interval (1.16–25.52), which shows that the connection is strong.

Table 1 Association analysis of MYBPC3 gene variant in the population of J&K

Gene	**Genotypic frequency**		**Allelic frequency**		**p-value**	**Odd Ratios**	**95% CI**
MYBPC3	**CASES**	**CONTROLS**	**CASES**	**CONTROLS**			
	90	98	W–0.90	W– 0.98	0.03	5.4444	1.16 - 25.52
	10	2	D –0.10	D – 0.02			

Table 2: Demographic and Life-style Factors of cases and controls

Variables	Characteristics	Cases (N=100)	Controls (N=100)	p-values
Age	Mean ±SD	55.86 ± 6.86	56.99 ±10.64	0.06
BMI	Mean ±SD	23.62±3.83	22.42±3.41	0.02
Gender	Male	66%	58%	-----
	Female	34%	42%	
Diet	Nonvegetarian	56	24	0.00
	Vegetarian	44	76	
Smoke	No	37	93	0.00
	Yes	55	7	
	Occasionally	8	0	
Alcohol	No	29	90	0.00
	Yes	58	10	
	Occasionally	13	0	
Tobacco	No	30	71	0.00
	Yes	52	4	
	Occasionally	18	25	

Demographic Profile

In the present study, 200 participants in total 100 cases and 100 controls were divided into a 1:1 ratio. The controls' mean age was 55.86 ± 6.86 years, whereas the cases' mean age was 56.99 ± 10.64 years. According to a t-test comparison, there was no statistically significant age difference between the two groups ($p > 0.05$), indicating that both groups' age distributions were comparable shown in Table 2. The study's patient population was composed of 34% female patients and 66% male patients. On the other hand, 58% of the controls were male, and 42% were females.

Lifestyle factors

Patients' BMI (20.62 ± 3.83 kg/m2) was significantly greater than that of healthy controls (18.42 ± 3.41 kg/m2), with a p-value < 0.002, indicating that elevated BMI may play a part in the development or susceptibility to disease. Diet, smoking, drinking alcohol, and using tobacco were among the lifestyle characteristics that were analyzed in both cases and controls. Among cases 56% of the patients were non-vegetarian, while 24% of the controls ate non-vegetarian. In contrast, 44% of the controlled group were vegetarians, whereas 76% of the patients followed a vegetarian diet. Smoking behaviors differed considerably between the two groups. The metabolic or inflammatory variables that affect disease risk may be influenced by this food habit. Cases had a greater smoking rate (55%) than controls (7%). Additionally, 8% of individuals were classified as occasional smokers in cases only. The results showed significant differences in smoking habits across groups ($p = 0.00$*). Alcohol intake has been assessed as a lifestyle factor in both cases and controls. Alcohol intake was more prevalent among patients (58% frequent users, 12% occasional) than in controls (10% regular, no occasional), with a significant p-value of 0.02. Tobacco usage and patient status were shown to be significantly correlated ($p = 0.00$). Only 4% of healthy people reported regularly using tobacco, compared to 52% of cases. In addition, controls were more likely to use it occasionally than cases group (25% and 18%). The association between tobacco use and a higher risk of disease is confirmed by this observation (Table 2).

4. DISCUSSION

In this study, the MYBPC3 mutation revealed a substantial correlation with cardiomyopathy in the Jammu population. Although cardiomyopathy has a poor survival rate and limited options for early detection or treatment, understanding its

causes might help prevent it. Our research revealed that the Jammu region's residents are at higher risk of disease than healthy people to have a higher BMI eating a non-vegetarian diet and smoking, drinking, and using tobacco products. Our findings are consistent with other research that found cardiac issues are more likely to occur in those who smoke and drink.

Numerous research studies have provided insights into the relationship between *MYBPC3* polymorphisms and the risk of cardiomyopathy; the results have varied depending on the population. It has been demonstrated that various groups have varying minor allele frequencies of genetic polymorphisms associated with cardiomyopathy (Golbus et al., 2012). In the South Asian population, the most reported mutation associated with hypertrophic cardiomyopathy (HCM) is a 25-base pair deletion (MYBPC3Δ25bp, rs36212066) in intron 32 of the MYBPC3 gene (Sadayappan et al., 2020). About 4% to 6% of people of South Asian heritage have this deletion, which translates to about 100 million carriers globally. A higher incidence of cardiomyopathies, such as heart failure, dilated cardiomyopathy (DCM), and HCM, has been linked to the *MYBPC3Δ25bp* variation (Arif et al., 2020). Recent research, however, has reexamined the *MYBPC3Δ25bp* variant's pathogenicity. According to Harper et al. (2020), there is no clear correlation between this prevalent variation and an elevated risk of HCM. Rather, it acts as a stand-in for MYBPC3 c.1224-52G>A, an uncommon, harmful intronic mutation that is linked out of equilibrium with *MYBPC3Δ25bp*. This uncommon haplotype (*MYBPC3Δ25bp*/c.1224-52G>A) has a high penetrance and is closely linked to HCM. MYBPC3 mutations were shown to be the second most common mutations causing illness in a multi-center European investigation that included 639 people with familial or sporadic DCM. Moreover, 13.8% of individuals with a variety of hereditary cardiomyopathies, such as restrictive cardiomyopathy, DCM, and HCM, have the MYBPC3Δ25bp deletion, which was first connected to HCM in two families (Waldmüller et al., 2003; Wessels et al., 2015).Our results are consistent with those of Geske et al., who found that the BMI of males and females differed statistically significantly ($p = 0.0053$) (Geske et al., 2017). Even though the difference they saw was little, it nevertheless supports the idea that BMI is an important consideration. Lakdawala et al., 2021 on the other hand, discovered no discernible variation in BMI between males and females ($p = 0.72$). Our findings, however, underline the importance of BMI in connection with illness and its possible influence on disease development and risk. Smoking cigarettes damages the cardiovascular system and has a role in the development of cardiomyopathy. Tobacco smoke's harmful ingredients cause myocardial cell death and structural alterations, which impair heart function. Chronic exposure to tobacco smoke causes "smoke cardiomyopathy," which is typified by degenerative cardiac lesions brought on by metabolic chemicals or poisons directly, as well as by alterations in blood flow that result in chronic hypoxia (Leone et al., 2008). Additionally, smoking changes the balance between coagulation and fibrinolysis and increases platelet aggregation, which both contribute to prothrombotic conditions. These alterations raise the possibility of thrombotic episodes, which can worsen myocardial injury and aid in the emergence of cardiomyopathy (United States. Public Health Service. Office of the Surgeon General, & United States. Office on Smoking. 2004). A well-known cause of alcoholic cardiomyopathy, which is represented by dilated ventricles and compromised cardiac contractility, is chronic excessive alcohol use. Alcohol's harmful effects on cardiac myocytes cause fibrosis, ventricular dysfunction, and cellular death. However, there is a complicated link between alcohol use and cardiomyopathy. While excessive alcohol use is harmful, some research indicates that modest to moderate alcohol consumption may have cardioprotective benefits. It is yet unclear how much alcohol is needed to cause cardiomyopathy, and each person is susceptible differently. To establish acceptable intake thresholds, more study is required, and care should be used when interpreting these results (Andersson et al., 2022)

Cardiomyopathy is a complex illness that is impacted by both genetic susceptibility and lifestyle choices. The fact that MYBPC3 polymorphisms vary in frequency and pathogenicity throughout populations highlights the complex nature of its genetic architecture. These results emphasize how crucial it is to take population-specific genetic variations into account when determining illness risk. Furthermore, to accurately determine the functional significance, possible harmful effects, and impact on the progression of disease and treatment response of both common and uncommon variations, thorough genomic analyses are required. At the same time, non-genetic variables like alcohol, tobacco, and smoking have a big impact on heart health and can either cause or exacerbate cardiomyopathy on its own or in combination. Chronic alcohol use is associated with dilated cardiomyopathy through direct myocardial toxicity and structural remodeling, whereas smoking and tobacco exposure cause cardiac damage through pathways including oxidative stress, hypoxia, and thrombotic processes.

Strengths, Limitations, and Future Directions

This study offers significant insights into an underrepresented demographic by examining the population from the Jammu area. The inclusion of both cases and controls enhances the study's dependability, and the use of PCR guarantees precise genotyping. The 200-person sample size, however, restricts its wider application; additional variables including environment and lifestyle should be considered. Future studies should examine gene-environment interactions and use more varied cohorts and greater sample sizes. Establishing causal relationships between the risk of cardiomyopathy and genetic variants will require long-term research. These revelations collectively highlight the complex etiology of cardiomyopathy and the necessity of an integrated strategy that incorporates lifestyle changes and genetic screening. Strategies for disease prevention and treatment must include specific genetic risk accounts and public health interventions aimed at reducing dangerous habits like alcohol and tobacco use. The goal of future studies should be to clarify the complex gene-environmental relationships that control the pathophysiology of cardiomyopathy to develop more accurate and potent treatment plans.

5. CONCLUSION

This study addressed *MYBPC3* genetic deletion variants in connection to cardiomyopathy and certain lifestyle variables. A substantial connection was discovered, with the variation indicating a strong relationship to increased cardiomyopathy risk in particular genetic models. A comparison was made between 100 healthy controls and 100 patients' lifestyle and behavioral traits. All factors showed statistically significant differences. However, more study with larger cohorts is needed to verify these findings and investigate the combined effect of genetic and environmental variables on cardiomyopathy risk.

Abbreviation

MYBPC3 - Myosin Binding Protein C

Δ25bp – 25 base pair deletion

PCR - Polymerase Chain Reaction

OR - Odds Ratio

CI - Confidence Interval

BMI - Body Mass Index

OPD - Outpatient Department

IEC - Institutional Ethics Committee

Statement of Ethics & Consent to Participate:

This study followed ethical guidelines and was approved by the Institutional Ethics Committee of Government Medical College Jammu (Ref: IEC/GMC/2022/1143). All participants gave written informed consent before taking part.

Conflict of Interest: The authors declare that there is no conflict of interest.

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Data Availability: All the data used in this study are included in the article. If you need more information, you can contact the corresponding author.

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PK and NB conceptualized the study and provided overall supervision. DK was responsible for patient diagnosis, while SB and PK compiled and organized the data. SB carried out the statistical analysis and interpreted the results. SB drafted the initial manuscript. Both SB and SP edited the figures and tables. PK, and NB revised the manuscript for intellectual content and finalized the manuscript. All authors reviewed the drafts, provided critical feedback, and approved the final version.

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