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Research Paper

Molecular Genetic Analysis of 25 bp deletion in *MYBPC3* Gene in Karachi, Pakistan

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ABSTRACT

This study focuses on South-Asian-specific 25-bps deletion in the *MYBPC3* gene which is linked to hypertrophic cardiomyopathy (HCM), a cardiovascular disorder characterized by heart muscle thickening and impaired function. Using 100 blood samples from healthy individuals in Karachi, Pakistan; we conducted a molecular genetics analysis of 25-bps deletion variant in the *MYBPC3* gene. Among the samples tested, 3 exhibited a homozygous deletion (genotype frequency = 0.1 or 10%), and 10 displayed heterozygous deletion (genotype frequency = 0.03 or 0.3%). Two participants with homozygous deletion were smokers, whereas one of these carriers had a family member with cardiomyopathy. The discovery of the 25-base pair deletion in the *MYBPC3* gene among healthy individuals calls for additional research to explore its potential clinical and therapeutic significance.

KEYWORDS: Cardiomyopathy, Homozygous, Hypertrophic, *MYBPC3*

INTRODUCTION

Cardiomyopathy encompasses a variety of heart muscle conditions with diverse causes, symptoms, and treatments, affecting people of all ages and races [1]. These diseases weaken the heart muscle's ability to pump blood, leading to irregular heartbeats, blood back up into the lungs or body, and heart failure. Cardiomyopathy can be acquired due to another disease or condition, or it can be inherited. The main types of cardiomyopathies include [1-2]:

Dilated Cardiomyopathy is characterized by the dilation of one of the heart's ventricles. This form is more common in males and is the most prevalent type in children. It can occur at any age and may be inherited or acquired.

Hypertrophic Cardiomyopathy (HCM) involves thickening of the heart muscle and often appears in childhood or early adulthood. It can cause sudden death in young athletes and is typically inherited. Family members with a history of HCM can be tested to reduce their risk of sudden death and irregular heartbeats.

In **Restrictive Cardiomyopathy,** the heart muscle becomes stiff or scarred, which can occur due to conditions like amyloidosis or hemochromatosis. This type is the least common.

Mutations in the cardiac myosin binding protein C (MyBP-C), encoded by the *MYBPC3* gene, are the most common cause of familial hypertrophic cardiomyopathy (HCM), an autosomal dominant inherited condition with incomplete penetrance. MyBP-C is crucial for normal cardiac function, binding strongly to the myosin filament backbone and modulating actomyosin sliding for muscle contraction. Heterozygous mutations in *MYBPC3* typically cause hereditary HCM, while homozygous frameshift mutations result in a severe, often lethal form of HCM in early infancy [3,12].

HCM is characterized by varying degrees of diastolic dysfunction, left ventricular wall thickening, progressive heart failure, and sudden cardiac death. A significant consequence of HCM is atrial fibrillation (AF), affecting 20 to 30% of individuals and

leading to higher risks of mortality, sudden death, and heart failure [4].

The primary aim of this study was to conduct a molecular genetics analysis of the South Asian-specific polymorphic *MYBPC3* 25 bps deletion variant in intron 32 in Karachi, Pakistan.

MATERIALS AND METHODS

We collected peripheral blood samples from the participants after informed consent. A total of 150 participants in the age group of 18 to 25 provided 2 ml blood samples. The participants were provided with comprehensive information about the procedure. The participant completed a consent form for their clinical information before the blood samples were drawn. The collected blood samples were carefully stored at -20 ℃ in a sterile EDTA-containing tube, appropriately labeled to maintain the participant's confidentiality.

The blood samples were used to test Activated Partial Thromboplastin Time (aPTT). The GJC DNA purification kit (Gene Janch Center, Karachi, Pakistan; genejanch.com) was used to extract and purify the DNA from these samples.

Using the genomic DNA, we employed PCR to amplify the specific region in the *MYBPC3* gene using forward primer (5'-GTTCCAGCCTTGGGCATATG-3') and reverse primer (5'-GAGGACAACGGAGCAAAGCCC-3'). The subsequent analysis of the resulting PCR amplicons by gel electrophoresis unveiled distinct band patterns that provided critical genetic information about the participants [10].

RESULTS AND DISCUSSION

In this study, we carried out a preliminary analysis of the occurrence of 25-bps deletion in *MYBPC3* gene in healthy individuals in Karachi, Pakistan.

In figure 1, the electrophoresis gel shows separation of DNA fragments, we differentiate bands denoted by letters H, M and N. The letter 'H' signifies the heterozygous deletion of 25 bps. The lane 'M' marks homozygous deletion of 25 bps, while lanes 'N' designates the normal.

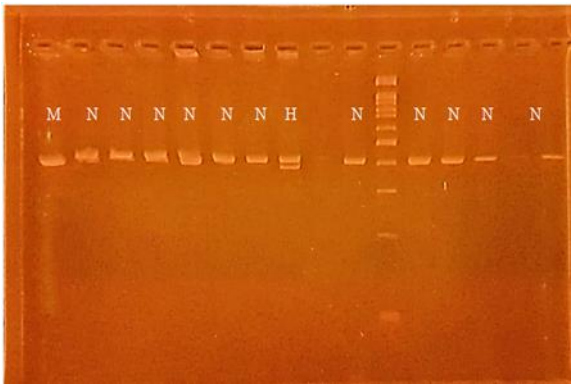

Figure 1: PCR amplicon bands indicating occurrence or absence of 25 bps deletion in intron 32 of *MYBPC3* gene. 'N' = no deletion; 'H' heterozygous deletion and 'M' homozygous deletion.

The cohort comprised 100 samples (42 females and 58 males). Among the participants tested, 3 exhibited homozygous 25 bps deletion (genotype frequency = 0.03 or 0.3%), and 10 displayed heterozygous 25 bps deletion (genotype frequency = 0.1 or 10%) in the *MYBPC3* gene. The minor allele frequency of homozygous deletion was calculated as 0.08. We also analyzed smoking habits and familial disease backgrounds. According to the clinical data forms, we found that nine female participants were smokers, while eighteen males identified as smokers. Looking at family disease history, four females and ten males had a background of cardiovascular disorders in their families. The total occurrence of deletion in *MYBPC3* was found in four males and eight females. Two females showed a connection between cardiovascular disease and smoking: same in the case of males.

The blood samples subjected to Activated Partial Thromboplastin Time (aPTT) as well. The Activated Partial Thromboplastin The

aPTT test assesses blood coagulation. It measures the time it takes for blood to clot after specific activators are added to a blood sample. This test provides additional information cardiovascular condition of the participants. For example, some forms of cardiomyopathy may be associated with an increased risk of thromboembolic events (such as blood clots), which could potentially influence aPTT results. Additionally, certain medications used to manage cardiomyopathy, such as anticoagulants or antiplatelet agents, may affect aPTT levels [5-7].

Most of the samples showed aPTT levels within the normal range. These results provide valuable insights into potential genetic variations within the population under study and prompt further exploration into their implications for thrombotic disorders and related health outcomes [8-9,11].

In conclusion, our approach provides a robust framework for genetic analysis of *MYBPC3* gene and informs clinical decision-making in the management of cardiomyopathies.

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