STUDIES ON GENE POLYMORPHISM AND NON-GENETIC FACTORS ASSOCIATED WITH CARDIOMYOPATHY PHENOTYPES IN THE JAMMU REGION OF JAMMU AND KASHMIR

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Zoology

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DECLARATION

I hereby declared that the presented work in the thesis entitled "**STUDIES ON GENE POLYMORPHISM AND NON-GENETIC FACTORS ASSOCIATED WITH CARDIOMYOPATHY PHENOTYPES IN THE JAMMU REGION OF JAMMU AND KASHMIR**" in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of Dr. Najitha Banu, working as Associate Professor, in the Department of Zoology/ School of Biosciences and Bioengineering of Lovely Professional University, Punjab, India and co-supervision of Dr. Parvinder Kumar working as Associate Professor, in the Department of Zoology and Deputy Coordinator Institute of Human Genetics of University of Jammu. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "**STUDIES ON GENE POLYMORPHISM AND NON-GENETIC FACTORS ASSOCIATED WITH CARDIOMYOPATHY PHENOTYPES IN THE JAMMU REGION OF JAMMU AND KASHMIR** " submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the **Department of Zoology/School of Biosciences and Bioengineering**, is a research work carried out by **Shikha Bharti**, Registration number **42100155**, is Bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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ABSTRACT

Abstract: Cardiomyopathy represents a clinically heterogeneous group of myocardial disorders influenced by both genetic and non-genetic (environmental) factors. Cardiovascular genomics has developed, even though limited is known about genetic correlations in many ethnic groups. This is the first study which investigates the genetic link to the cardiomyopathy in the Jammu region. The study was carried out in the Union Territory of Jammu and Kashmir, India. A key factor in determining the phenotype is how the causative mutations affect the structure and functionality of the corresponding proteins. For instance, *MYH7* mutations are linked to a comparatively greater frequency of sudden cardiac death, substantial hypertrophy, and an early start. *MYBPC3* mutations, on the other hand, are linked to moderate heart hypertrophy that develops later in life and delayed clinical manifestation. The phenotype of cardiomyopathy can also be changed by non-molecular variables such environmental influences, nutritional practices, alcohol use, physical activity, hypertension, cardiac loading circumstances, and obesity. Heart disease is caused by several interrelated biological processes, each of which has a different impact on the result of the disease. Despite considerable advances in global understanding of the genetic and nongenetic causes of cardiomyopathy. But there is less information that is known about the Jammu region. Very few research was conducted on the genes associated with cardiomyopathy and their effects on the specific population. This demonstrates a substantial research gap in this Jammu region's understanding of cardiovascular genetics.

The primary goal of this case-control investigation was to examine the relationship between susceptibility to cardiomyopathy and the selected candidate gene polymorphisms. The genes selected for the current study are cardiac myosin binding protein C (*MYBPC3 Δ25bp*), β-myosin heavy chain (*MYH7 G>A*), and angiotensin converting enzyme (*ACE I/D*) and *BAG 3* gene. Additionally, the cumulative effect of these genetic variants and non-genetic contributors was to be analysed. Apart from genes linked to cardiomyopathy, this study also identifies the telomere length association with the risk of cardiomyopathy. Telomere shortening has been connected to several age-related illnesses, including atherosclerosis, diabetes, heart disease and cancer. It has been discovered that genes that maintain the health of telomeres may contribute to the development of cardiomyopathy.

The present study evaluated the association of four candidate gene polymorphisms with the susceptibility of cardiomyopathy as well as the impact of risk alleles and possible environmental factors on the risk of disease in a case-control manner. A total of 750 participants were recruited, including 500 age-matched healthy controls and 250 patients with clinically proven cardiomyopathy. *ACE I/D* and *MYBPC3 Δ25bp* polymorphisms were genotyped using polymerase chain reaction (PCR) whereas *MYH7 (G>A)* polymorphism was examined using PCR-restriction fragment length polymorphism (PCR-RFLP) and *BAG3* polymorphism was done by using TaqMan SNP genotyping Assay (qPCR). The case and the control groups were compared in terms of genotypic and allelic frequencies, and their relationships were assessed using chi-square tests, odds ratios (ORs), and 95% CI by using SPSS version 25. To evaluate the significance of age match controls, a t-test was implemented. The different biochemical parameters such as cholesterol, triglycerides, HDL, sodium, potassium, urea SGOT, SGPT suggested a significant association with cardiomyopathy. In addition, increased caffeine intake (OR=213.32), diet (OR=28.66), alcohol consumption (OR=89.99), smoking (OR=166.45) and tobacco (OR=229.04) showed significant association with the disease. For genetic factors, cardiomyopathy was significantly associated to the *ACE I/D* polymorphism as patients had a greater frequency of the D allele (50.6%) than controls (42.9%). Carriers of the D allele were found to have one-fold increase the risk of disease with an odd ratio (OR = 1.36) and p value 0.0048. There was also a high correlation with the *MYH7 (G>A)* polymorphism, with the A allele being more common in cases (33%) compared to controls (23%) and p= 0.0002 values show significant association of A allele with the disease having an odd of 1.68 at 95% CI: 1.32-2.14. The *MYBPC3 Δ25bp* deletion allele was also found in cases (29%) much more frequently than in controls (5.2%), suggesting a seven folds greater risk than those with the wild allele G. (OR =7.374; 95% CI: 4.56-11.92; p = 0.000). Furthermore BAG-3 gene also associated with the C allele frequency is higher in cases (40.2%) compared to controls (26%) and yielding a high odds ratio of 1.91 (95% CI: 1.52-2.40; $p < 0.0001$). These results underscore the extent to which risk alleles contribute to the development and progression of cardiomyopathy. It also demonstrates the significance of gene-gene interactions, specifically those between modifier genes and sarcomeric or cytoskeletal genes, in the etiopathogenesis of cardiomyopathy using the Multifactor Dimensionality Reduction (MDR) framework. The study indicates that these interactions contribute to phenotypic variance in CM, especially when considered in

combination with epidemiological parameters such as age, sex, and environmental exposures. The telomere length deterioration is higher in cases than in controls and these findings highlight the role of telomere with the disease. Also, it is significantly influenced by age, gender and BMI.

Thus, the present research investigation observed an elevated risk of cardiomyopathy in the Jammu population, with polymorphisms in the *ACE*, *MYH7*, *MYBPC3* and *BAG-3* genes. This study also illustrates the link between the shortening of telomeres with the risk of developing cardiomyopathy. The chance of developing cardiomyopathy was proven to be synergistically increased by the cumulative presence of risk alleles across these genes, indicating a polymorphic significance. Furthermore, these genetic variations may interact with non-genetic variables including lifestyle, environmental stresses, and associated illnesses to modify the development and course of disease. The results highlight the significance of gene-gene interactions in the pathogenesis of cardiomyopathy and provide new perspective into the genetic landscape of the disease in the Jammu population. It is anticipated that a comprehensive examination of telomere biology in the pathophysiology of cardiovascular disease would increase our knowledge of the genetic components of the disorder and reveal new genes and pathways that contribute to its development.

Further, this current research indicates the need for more large-scale research, functional validations, and investigation of gene-gene interactions while providing important information on the genetic architecture of cardiomyopathy in an underrepresented community. It is expected that the development of high-throughput, rapid and extremely precise mutation detection technologies would greatly expand the range and accuracy of genetic testing and diagnostics. In addition to helping identify new pharmaceutical targets and individualized management plans for cardiomyopathy, these studies are crucial for clarifying the underlying molecular pathways.

Keywords: polymorphism, Jammu, cardiomyopathy, *ACE*, *MYH7*, *MYBPC3*, *BAG3*

Dedicated

in the loving

memory of

my brother

Shivam Sharma

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ABBREVIATIONS/ACRONYM

CM	Cardiomyopathy
DCM	Dilated Cardiomyopathy
HCM	Hypertrophic Cardiomyopathy
RCM	Restricted Cardiomyopathy
ARVCM	Arrhythmogenic Right Ventricular Cardiomyopathy
SNP	Single Nucleotide Polymorphism
CNS	Central Nervous System
MyBP-C	Myosin Binding Protein C
PCR	Polymerase Chain Reaction
FN-3	Fibronectin III
Ig	Immunoglobulin
LV	Left Ventricle
LVH	Left Ventricular Hypertrophy
MYH7	Myosin Heavy Chain Gene
MYBPC3	Cardiac Myosin Binding Protein-C3 gene
cMyBP-C	Cardiac Myosin Binding Protein-C
MYBPC3 Δ25bp	25 Base Pair Deletion Mutation in MYBPC3
cMyBP-CC10mut	Protein Product of MYBPC3 Δ25bp gene
IEC	Institutional Ethics Committee
EDTA	Ethylene-Diamine Tetra Acetic Acid
HC	Healthy Control
SDS	Sodium Dodecyl Sulphate
AHA	American Heart Association
WHO	World Health Organization
BAG3	Bcl-2–Associated Athanogene 3
ESC	The European Society of Cardiology
BV	Bi-Ventricular
EF	Ejection Fraction
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
SCD	Sudden Cardiac Death

TTN	Titin
LMNA	Lamin A/C
ACTC1	Cardiac Alpha Actin
SGCD	Delta-Sarcoglycan
SCN5A	Sodium Channel, Type V
DSP	Desmoplakina
MYL2	Myosin Light Chain 2
MYL3	Myosin Light Chain 3
TPM1	Tropomyosin 1
FLNC	Filamin C
BAG3	BLC2 - Associated Athanogene 3
MYBP-C	Myosin Binding Protein C
TNNT2	Cardiac Troponin T
TNNI3	Cardiac Troponin I
DES	Desmin
SGCD	Delta-Sarcoglycan
DMD	Dystrophin
SCN5A	Sodium Channel, Type V
MYH7	Myosin Heavy Chain gene 7
CVD	Cardiovascular Disease
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
LVH	Left Ventricular hypertrophy
ECM	Extracellular Matrix
LIM	Lin-11, Islet-1, Mec-3
VCL	Vinculin
MDR	Multifactor Dimensionality Reduction
qPCR	Quantitative Polymerase Chain Reaction
TL	Telomere Length
GMC	Government Medical College
SSH	Super Speciality Hospital
µg/ml	Microgram Per Millilitre

µl	Microliter
nm	Nanometer
U/L	Units per Liter
mg/dL	Milligrams Per Deciliter
mmol/L	Millimoles Per Liter
ng/µl	Nanogram Per Microliter
µM	Micromolar
rpm	Revolutions Per Minute
MgCl2	Magnesium chloride
%	Percentage

APPENDICES

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2.	Approval from the School of Biosciences and Bioengineering
3.	Consent and Questionnaire Form Cases
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CHAPTER-1
INTRODUCTION

1.1 Cardiomyopathy

Cardiomyopathy is a subacute or chronic disorder of the myocardium that causes abnormalities in cardiac wall thickness, chamber size, contraction, relaxation, conduction, and rhythm. It causes damage to the heart's skeletal muscles and reduces its capacity to circulate blood throughout the body. The term cardiomyopathy refers to impaired heart health conditions that have no association with rheumatic, hypertensive, coronary artery, thyroid, or congenital conditions as the primary causes. Thus, Cardiomyopathy is a medical assessment that involves complications with the structure or function of the heart muscle, identified by unusual size of the chamber and thickness of the wall or dysfunctional contractility, such as systolic or diastolic dysfunction. These conditions occur without any presence of congenital heart disease, coronary artery disease, valvular disease or hypertension. It is a diverse group of diseases that can lead to heart failure and other serious health issues. The signs and symptoms of cardiomyopathy are like heart failure, including fatigue, shortness of breath, sudden nighttime awakenings, cough, difficulty breathing while lying down, due to difficulty breathing, and swelling (Jang, 2019). Cardiomyopathies remain undiagnosed in early stages, but their symptoms resemble with heart failure, regardless of systolic or diastolic pressure. Heart failure has been associated with 20% mortality rate within one year and 70-80% within eight years (Weintraub et al., 2017; Groenewegen et al., 2020). There are two main categories of cardiomyopathies: Primary cardiomyopathy and secondary cardiomyopathy.

1.1.1 Primary Cardiomyopathy

Primary (comprising genetic, mixed, and acquired forms affect only or mostly the heart muscle) (Schultheiss et al., 2019). According to WHO studies from 2003, cardiomyopathy may be divided into four primary kinds according to its pathophysiology and anatomical features.

1.1.1.1 Dilated Cardiomyopathy

Dilated cardiomyopathy (DCM) is a widespread form of cardiomyopathy that involves the expansion and weakened contraction of one or both ventricles, which can occur because of primary factors like genetics or acquired factors like infiltration or autoimmune issues. The primary feature of dilated cardiomyopathy is abnormal

contraction of the left ventricle, which leads to an increase in mass and volume (Heymans et al., 2023). In certain cases, left ventricular diastolic abnormalities may also be present, and right ventricular dysfunction can also occur. The thrombus may form in the apices of the ventricles in the latter phase (Wexler et al., 2009). This illness may lead to heart failure and sudden cardiac death, leading to increased hospitalisations and potential need for heart transplantation (Jefferies and Towbin, 2010; Arrigo, 2020), which can be very costly. Dilated cardiomyopathy can develop in individuals of any age, sex, or ethnic origin (Maron et al., 2006; Heymans et al., 2023).

Dilated cardiomyopathy is more frequently observed in men among adults, whereas in children, it affects boys more often than girls, particularly in black individuals and babies under the age of one. The idiopathic form of the disease is estimated to affect two-thirds of children (Towbin et al., 2006; Malinow et al., 2024). The prevalence of the disease in adults is around one in 2500 individuals, with an average of seven per annum. However, the number could be higher, and it's mostly underdiagnosed, often inherited and known as familial dilated cardiomyopathy, which may account for 20 to 48% of all cases (Taylor, 2006; Amrein et al., 2023).

Adults commonly develop dilated cardiomyopathy because of ischemic cardiomyopathy caused by coronary artery disease and hypertension. However, other factors such as valvular disease, viral myocarditis, and genetic predisposition may also contribute to the condition (Maron and Towbin, 2006; Baggio et al., 2021). On the other hand, children with dilated cardiomyopathy usually experience it during their first year of life, and it is mainly caused by idiopathic myocarditis and neuromuscular diseases. Diseases affecting the neuromuscular system, like Becker muscular dystrophy, Duchenne muscular dystrophy, and Barth syndrome, a genetic disorder linked to the X chromosome, which involves skeletal myopathy, dilated cardiomyopathy, and neutropenia, are widely recognised as major contributors to health concerns (Kaski and Elliott, 2007).

1.1.1.2 Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is a genetic cardiac disorder that primarily affects the left ventricle and causes excessive thickening (hypertrophy) of the heart muscle. This thickening may lead to problems such as arrhythmias and

sudden cardiac death by impairing the heart's ability to pump blood efficiently (Basit et al., 2024). It is the most prevalent genetic cardiac conditions that affect around one in 500 people (Maron et al., 1995; van Driel et al., 2019). Gene disorders that create the heart's contractile proteins cause the heart muscle, especially the left ventricle and the interventricular septum, to thicken abnormally. Nonobstructive HCM happens when there is no obstruction, but obstructive HCM happens when the thickness prevents the heart from pumping blood (Raphael et al., 2016). Many HCM patients have no symptoms at all, and they may not even be aware that they have the condition. Nonetheless, some people may have symptoms including dizziness, shortness of breath, chest pain, palpitations, fatigue, or fainting, particularly when they are actively exerting themselves. Causative mutations can be detected with current genetic testing techniques in 30–60% of HCM patients; detection rates are higher in those with a positive family history (Ireland et al., 2024). The majority of HCM is mainly inherited as an autosomal dominant characteristic. It is caused by variations in sarcomere protein genes. Hypertrophic cardiomyopathy results from mutations in 11 genes, leading to over 500 distinct mutations (Wexler et al., 2009; Kambis, 2022). A major variation involves the beta-myosin heavy chain, a protein that binds myosin (Maron et al., 2003). The potential for nongenetic variables is increased by the variety and heterogeneity that might alter the phenotype of HCM. Patients with this condition often experience decreased exercise capacity and symptoms of chest pain and exertional dyspnea due to various factors such as mitral regurgitation, impaired relaxation, and compliance of the left ventricle, and obstruction of the outflow tract of the left ventricle, as well as microvascular dysfunction and subendocardial ischemia (Raphael et al., 2016; Reed et al., 2022)

1.1.1.3 Restrictive Cardiomyopathy

Restrictive cardiomyopathy (RCM) is a rare form of cardiomyopathy characterised by elevated rigidity of the ventricular walls without a corresponding increase in wall thickness. This rigidity impairs the ventricle’s ability to relax and fill enough during diastole, leading to abnormal ventricular function. As the disease progresses, valvular dysfunction may occur, the myocardial contractility declines, and the ventricles gradually lose their ability to effectively pump blood, ultimately leading to heart failure (Sen-Chowdhry et al., 2010). RCM may result from genetic mutations or infiltration of myocardial tissue (Tariq and Ware, 2014; Cimiotti et al., 2021).

Hemodynamically, it is defined by a significant increase in ventricular pressure with only a moderate increase in filling volume, due to increased myocardial stiffness. In several cases, restrictive physiology is present only during specific stages of disease progression either in the early phase before the onset of ventricular dilation and reduced contractility or in the late phase following a hypertrophic phenotype (Rapezzi et al., 2020). In clinical terminology, restricted cardiomyopathy is defined as having normal or decreased systolic and diastolic chamber volumes in one or both ventricles, but the ventricular wall thickness remains normal (Severino et al., 2021). Other pathological mechanisms associated with RCM include endomyocardial fibrosis, extracellular matrix infiltration, accumulation of fat and storage material within cardiomyocytes, interstitial fibrosis, and intrinsic myocardial dysfunction (Elliott et al.,2008).

1.1.1.4 Arrhythmogenic Right Ventricular Cardiomyopathy

Arrhythmogenic right ventricular Cardiomyopathy (ACM) is a rare inherited heart condition. It was first observed in young individuals who died suddenly while doing normal tolerance exercise (Davies, 2000). ACM is estimated to occur in 1 out of every 100 to 5000 individuals in the general population. However, there may be additional unreported cases of ACM, as unexpected cardiac death can occur as the first sign of the disease (Groeneweg et al., 2015; Ottaviani et al.,2021). ACM is characterised by the replacement of normal tissue with fibrotic and fatty tissue in the ventricular cardiomyocytes. It leads to systolic dysfunction and electrical instability within the right or left ventricle (Corrado et al., 2017; Bharti et al., 2024a), or both. ACM, like other forms of cardiomyopathies, has a wide range of genetic origins and is primarily caused by alterations in the genes that code for structural proteins (Asimaki et al., 2007; Gerull and Brodehl, 2021). When desmosomes malfunction, it can affect the communication between cells and can also cause cell death, leading to the replacement of cardiomyocytes with fat and/or fibrous tissue (Bergmann et al., 2009). ACM typically appears during adolescence or adulthood, and its symptoms include chest pain, palpitations, syncope, supraventricular arrhythmias, ventricular tachycardia, and right heart failure (Peters et al., 2004). The right ventricular inflow (from the tricuspid valve), outflow (towards the pulmonary artery), and apex (tip of the ventricle) frequently form a "triangle of dysplasia" (Elliott et al., 2008).

Nonetheless, an early diagnosis can postpone the onset of severe ACM (Hamilton and Fidler, 2009).

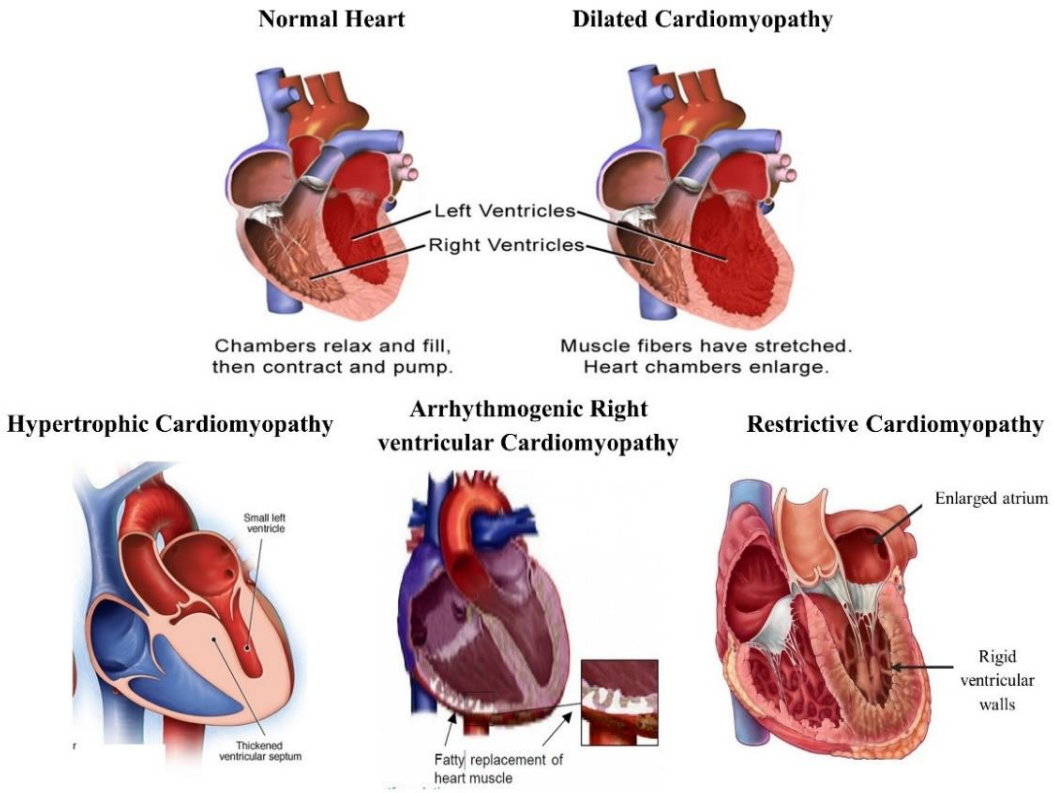


Figure 1.1: Types of Cardiomyopathies (Image by using Bio-Render) https://www.nhlbi.nih.gov/health/cardiomyopathy/types

1.1.1.5 Left Ventricular Non-Compaction (LVNC) Cardiomyopathy

It is believed that the disease originated from a disrupted development in the embryo, resulting in an enlarged ventricle with a sponge-like texture caused by irregular trabeculations. Some mutations have been discovered in certain genes, such as dystrophin-related protein family member, tafazzin, which play a critical role towards cardiolipin metabolism in mitochondrial membrane protein, dystrobrevin, and a gene that encodes lamin, found in the nuclear envelope (Moric and Markiewicz, 2008). LVNC is connected to left ventricular dysfunction and serious arrhythmia. There is a high thrombus formation risk in the trabeculae, which can cause embolic stroke or sudden cardiac death (Cimiotti et al., 2021).

1.1.1.6 Takotsubo Cardiomyopathy

Takotsubo cardiomyopathy, also referred to as stress-induced cardiomyopathy, is a condition that results in sudden left ventricular dysfunction as a reaction to severe

emotional or physiological stress (Maron et al., 2006). This condition is more frequently observed in postmenopausal women, and its prevalence is challenging to determine, though it is estimated to affect approximately 0.02% of hospitalised patients. Takotsubo cardiomyopathy may account for 1-2% of acute coronary syndrome hospitalisation cases (Deshmukh et al., 2012; Butt et al., 2022). Elevated cardiac enzymes may be observed in laboratory tests, and initial treatment is accordingly provided for acute coronary syndrome (Bhatt et al., 2022).

1.1.2 Secondary Cardiomyopathies

Secondary cardiomyopathy refers to heart muscle disease that results from non-cardiovascular causes and multiorgan or systemic diseases that result in damage to the myocardium (Richardson, 1996; Maron et al., 2006). Specific disease patterns can be associated with certain aetiologies, such as alcohol consumption resulting in expanded morphology or amyloidosis triggering restrictive physiology. However, the display of pathology resulting from systemic disease can differ. Secondary reasons can be classified into nutritional, toxic, endocrine, autoimmune, and neuromuscular groups (Luk et al., 2009). The primary objectives of assessment and treatment are to deal with the fundamental disease process, eliminate any harmful agents, and regulate symptoms of heart failure (Maron and Towbin, 2006). Secondary cardiomyopathies are caused by an underlying factor, such as arterial hypertension, heart valve disorders, or metabolic challenges. This can result in the less ejection fraction and hypertrophy of the left ventricles (Chugh et al., 2008; Santos and Shah 2014). In Diabetic cardiomyopathy, high blood sugar levels damage the heart muscle and impair its function. This can lead to both diastolic and systolic dysfunction, as well as fibrosis and inflammation (Jia and Hill, 2019). In Toxin-induced cardiomyopathy, exposure to toxins such as alcohol, cocaine, chemotherapy or treatment drugs can cause disease. The effects are driven by toxin-induced oxidative damage, inflammation, and mitochondrial abnormalities, which damage the heart muscle and impair its function (Kim and Januzzi, 2021). In inflammatory cardiomyopathy, conditions such as myocarditis or systemic lupus erythematosus can also cause secondary cardiomyopathy. In these cases, the inflammation damages the heart muscle, leading to dysfunction and fibrosis (Kishimoto et al., 2019). A variety of underlying diseases and factors can cause secondary cardiomyopathies. It is important

to identify and treat the underlying causes to prevent further damage to the heart muscle and improve outcomes for patients.

1.2 Prevalence of Cardiomyopathy

1.2.1 Prevalence of Cardiomyopathy Worldwide

Heart disease is one of the main health problems worldwide, affecting millions of lives and leading to a high number of deaths, significantly contributing to disability and loss of productivity, which places a substantial burden on both individuals and society (Dominguez et al., 2016) Among various forms of cardiovascular disease (CVD), cardiomyopathy is a relatively uncommon yet serious condition characterised by structural abnormalities of heart, arrhythmias, and heart failure (Tschope et al., 2020). Despite being a major health concern, the overall prevalence of cardiomyopathy remains uncertain, as it can affect individuals of any age, sex, or ethnic background (Sisakian et al., 2014). The reported incidence and prevalence vary greatly depending on the populations and geographic locations that were researched. Due to the disease's very low incidence and the dearth of extensive, reliable epidemiological investigations, the existing data are frequently underestimated. Based on a study that was carried out in Minnesota, USA, between 1975 and 1984, and involved 46 people with idiopathic DCM, the first-ever report on DCM prevalence was published. According to this study, the prevalence was 36.5 cases per 100,000 people, and the incidence rate was 6.0 cases per 100,000 people after following sex and age adjustments.

In 2015, the Global Burden of Disease study estimated approximately 2.5 million cases of cardiomyopathy worldwide, marking a 27% increase over a decade. By 2016, the number of heart failure cases had risen to approximately 37.7 million, and by 2017, this figure had reached 64.3 million, with 29.5 million men and 34.8 million females, the higher prevalence among women (Ziaeian and Fonarow, 2016). Males had a substantially higher age-standardised prevalence rate of alcoholic cardiomyopathy than females, with a rate of 27.7 versus 12.6 per 100,000 individuals (Dai et al., 2021).

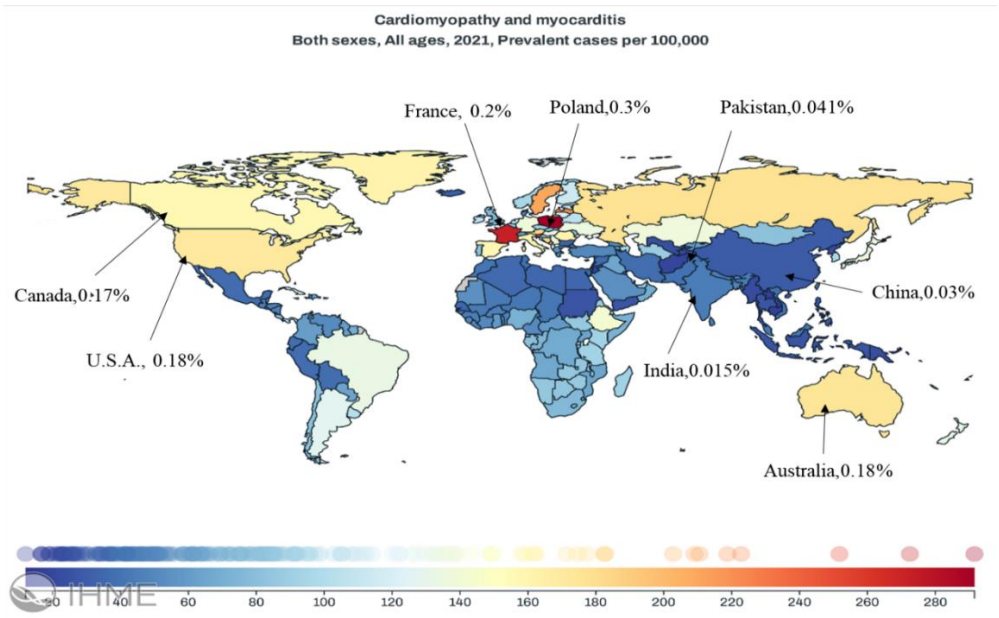


Figure 1.2: Worldwide Distribution of Cardiomyopathy (https://www.thelancet.com/lancet/visualisations/gbd-compare).

The Global Burden of Disease (GBD 2021) reports states, the prevalence of cardiomyopathy per 100,000 individuals varied significantly across countries, with the highest rates observed in Poland (291.5), France (272.49), and Sweden (241.04), Canada (158.43), while lower rates were reported in China (34.49), Pakistan (38.96), and India (43.88). Among different types, dilated cardiomyopathy (DCM) is the most prevalent, affecting over 1 in 250 individuals, with higher occurrence in men than women and an incidence rate of 0.57 per 100,000 in children, predominantly in boys (0.66 vs. 0.47 per 100,000, $P < 0.006$). Hypertrophic cardiomyopathy (HCM) affects approximately 1 in 500 individuals, with a prevalence rate of 0.2%, predominantly occurring in males aged 55 to 64 years (Butzner et al., 2022). Restrictive cardiomyopathy (RCM) is rare but constitutes about 5% of all cardiomyopathy cases, with regional variations influenced by underlying causes such as endomyocardial fibrosis in tropical regions and cardiac amyloidosis in other areas (Beaton et al., 2017). Arrhythmogenic right ventricular cardiomyopathy (ARVC) has an estimated prevalence of 1 in 2,500 to 1 in 5,000 individuals. It is responsible for 5% to 10% of sudden unexplained deaths in those under 65, occurring more frequently in men than women at a ratio of 2.7:1. The prevalence of ARVC is higher in certain regions, particularly in Italy and Greece, where it ranges between 0.4% and 0.8% (McNally et al., 2005). Genetic, environmental, and socio-economic factors influence the

occurrence of cardiomyopathy, with specific forms such as Chagas disease-related cardiomyopathy being more common in Latin America. Lifestyle choices, including alcohol consumption, obesity, and hypertension, as well as medical conditions like diabetes and viral infections, further contribute to its prevalence. Given its association with heart failure and sudden cardiac death, cardiomyopathy remains a significant global health challenge. Continuous research is essential to better understand its epidemiology and develop effective early diagnosis, treatment, and prevention strategies.

1.2.2 Prevalence and Incidence of Cardiomyopathy in India

In India, the prevalence of dilated cardiomyopathy (DCM) has been rising steadily over time. A consistent rising trend was evident from the roughly 106,460 cases that were recorded in 1990 to the 150,507 additional cases that were reported by 2005. The disease's rising prevalence was demonstrated in 2019 when 207,168 new cases were reported, continuing this upward trend (Sarohi et al., 2022).

In India, RCM is not idiopathic instead endomyocardial fibrosis is the primary aetiology of RCM. Females show a higher prevalence of idiopathic RCM than men (female: male ratio, 15:1) (Kapoor et al., 2017). In contrast to other Asian countries, more than half of Indians get arrhythmogenic right ventricular cardiomyopathy before the age of thirty, indicating that the sickness strikes at a fairly young age. A significant influence of genes was seen in the family histories of most early-onset individuals. (Mushtaque et al., 2020).

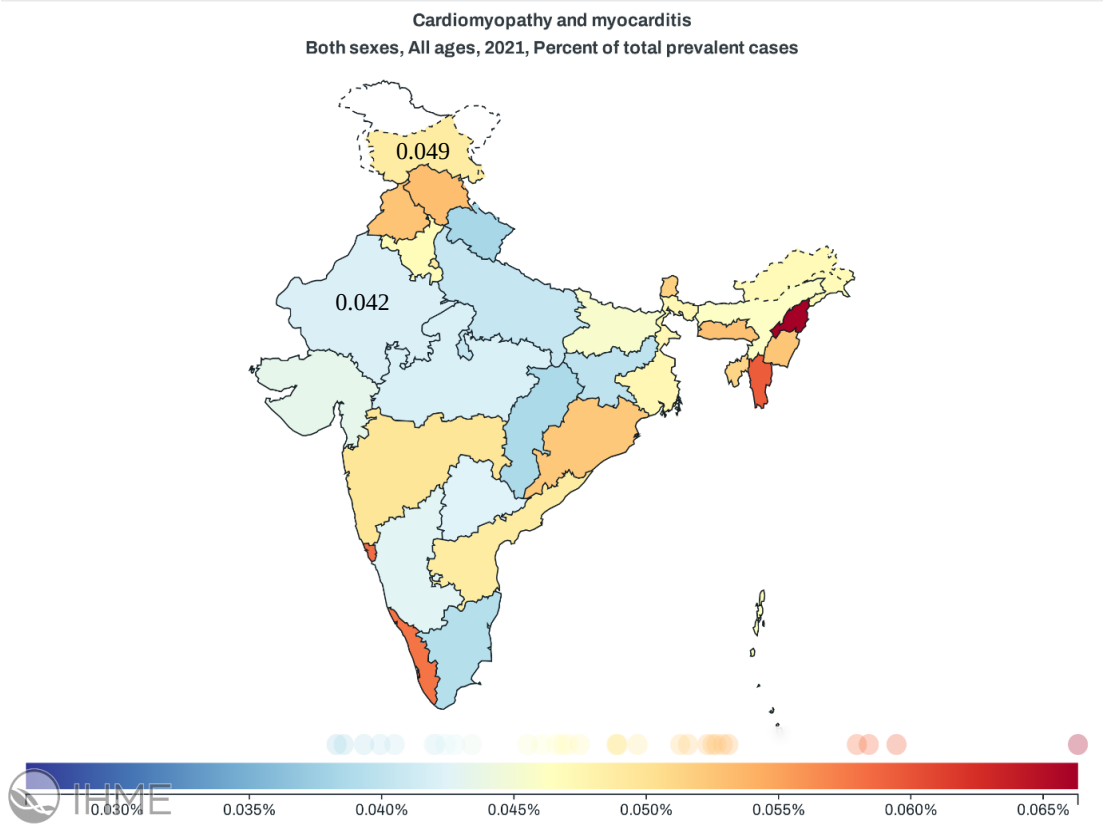


Figure 1.3: Distribution of Cardiomyopathy in India (https://www.thelancet.com/lancet/visualisations/gbd-compare).

According to Prabhakaran et al. (2016), the estimated cardiomyopathy mortality rate in India is 3.87 fatalities per lakh, significantly higher than the global average. Regional differences reveal that Punjab has a higher death rate of 4.63 deaths per lakh, while Jammu & Kashmir and Ladakh have a rate of 3.34 fatalities per 100,000. These variations point to variations in risk factors, disease management techniques, and healthcare access among states. As per the Global Burden of Disease (GBD) 2021, the incidence rate of cardiomyopathy is 15.39 new cases per 100,000 people per year, whereas the prevalence rate is 0.045 per 100,000 people in India. Among Indian States, Nagaland leads in prevalence, with 0.66 instances per 100,000, followed by Goa (0.058 cases per 100,000), Himachal Pradesh and Punjab (0.053 cases per 100,000), and Jammu & Kashmir (0.049 cases per 100,000).

The prevalence of cardiomyopathy in Jammu & Kashmir is 0.049 cases per 100,000 people, which is still a serious public health problem even though it is lower than in some other states. This increasing load may be caused by several factors, including lifestyle modifications, genetic predisposition, and restricted access to

expert cardiac care. Better healthcare facilities, early detection initiatives, and awareness campaigns are necessary to combat the increasing prevalence and strengthen regional prevention and treatment initiatives.

1.2.3 Prevalence in Jammu & Kashmir

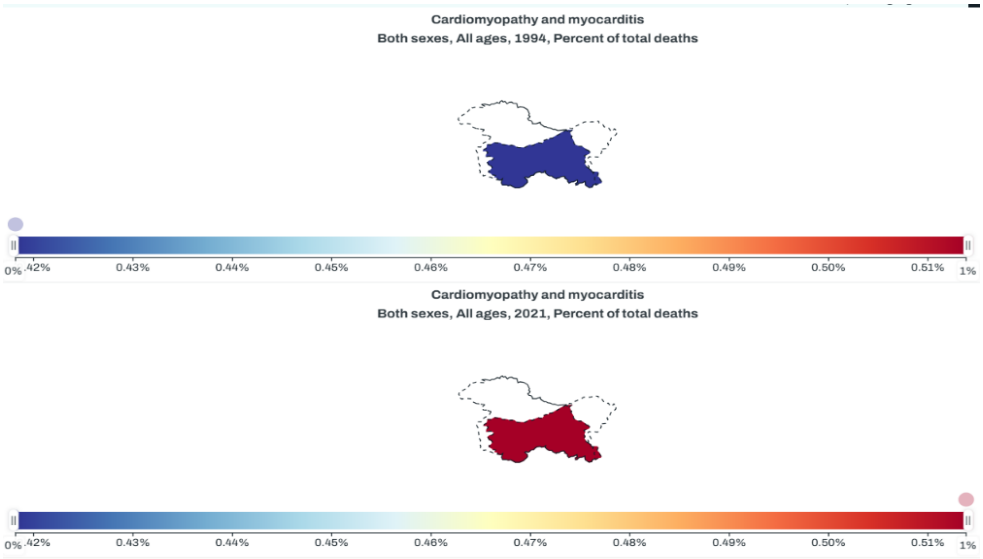


Figure 1.4: Comparison of Deaths by Age and Sex in 1994 and 2021 in J&K (https://www.thelancet.com/lancet/visualisations/gbd-compare)

It has been seen currently that in regional J&K, heart failure is seen primarily due to cardiomyopathy. Out of all kinds of cardiomyopathy, Dilated cardiomyopathy has shown more prevalence than hypertrophic cardiomyopathy, followed by restrictive and arrhythmogenic cardiomyopathy in sequence. But dilated and hypertrophic cardiomyopathy was mostly seen in males than in females (Shairgojri et al., 2021).

1.3 Risk Factors

A risk factor is defined as a characteristic, behaviour, or environmental exposure that increases the likelihood of developing a disease. In the context of cardiomyopathy, risk factors are broadly categorised into genetic and non-genetic types. The development of cardiomyopathy involves a complex interplay between genetic, environmental, and acquired factors.

Figure 1.5: Systematic Representation of Risk Factors

1.3.1 Non-Genetic Risk Factors

Age: The function of the heart is mainly influenced by age because with an increase in age, the elasticity of the muscle decreases. The ability of the heart to work in pressure (compliance) of the arterial system also decreases with age. This results in a decrease in pumping action of the heart, thereby increasing the workload to pump blood to the various organs of the body. Although cardiomyopathy can occur at any age, the prevalence of this disorder is higher in older people aged above 45years. (Tromp et al., 2001).

Gender: Gender-related differences in phenotypic expression and outcomes are a primordial risk factor of cardiomyopathy (Fairweather et al., 2021). Cardiomyopathy is reportedly higher in men and increases with age, while premenopausal women have been reported to have better long-term survival from cardiomyopathy. After many words, both men and women are equally affected by this disease (Fairweather et al., 2023).

Obesity: Obesity directly raises the risk of cardiomyopathy and heart failure due to diastolic dysfunction. The replacement of the muscle tissue in the right ventricle by fatty or fibrous tissue in patients with obesity, which causes disturbances in the electrical signals of the heart and dysfunction of atria and ventricles. But in severe situations, myocardial structural changes abruptly cause cardiac arrest (Bharti et al., 2024a).

Alcohol: Excessive alcohol consumption has been studied as a modifiable risk factor for heart diseases greater than 60 gram per day in men and greater than 40 gram per day in women is a well-known contributor to mortality and burden of cardiac disease (Hoek et al., 2022) When compared to moderate consumption, moderate to excessive

alcohol use negatively impacts cardiac function and muscle strength, resulting in increased LAVH, decreased biventricular function, and increased chamber dilatation (left atrium and ventricle, right ventricle). Alcohol increases hormones like cortisol that cause arteries to tighten and constrict, and increase the heart rate and BP (Andersson et al., 2017; Bharti et al., 2024a).

Tobacco/Smoking: The primary way that tobacco is smoked is in cigarettes. Health risks associated with tobacco include both direct cigarette consumption and indirect smoke exposure (Gallucci et al., 2020). Smoking causes irritation to the endothelial lining of blood vessels. Chemicals found in the smoke from cigarettes cause the blood vessel lining cells to swell and become inflamed. This could constrict blood vessels and cause a plaque to develop (Roy et al., 2017; Mahoney et al., 2021).

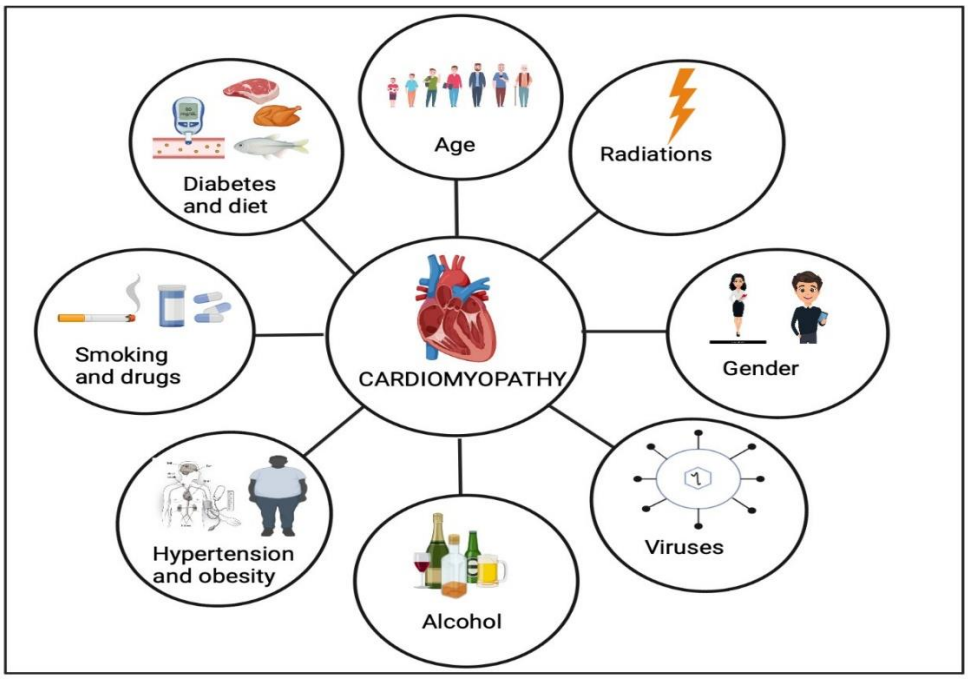


Figure 1.6: Representation of Non- genetic Factors of Cardiomyopathy

Diet: The increase in obesity, diabetes, hypertension, and other cardiovascular diseases (CVD) is mostly caused by inadequate nutrition (Lim et al., 2010). Cardiovascular risk is closely linked to diets that are low in fruits, vegetables, legumes, whole grains, and seafood and high in trans and saturated fats, refined grains, added sugars, and salt (Dong et al., 2022). Cardiomyopathy may occur because of these dietary patterns' promotion of atherosclerosis, which causes arterial stiffness and increased cardiac strain. Additionally, a clear connection has been

established between CVD and diabetes mellitus (DM). The primary cause of death and disability among diabetics is still cardiovascular disease (CVD). Because of the higher rates of myocardial infarction and stroke, adults with diabetes in the United States are at 1.7 times the risk of dying from CVD (Leon et al., 2015). Diabetic cardiomyopathy is a disorder characterised by structural and functional alterations in the heart muscle that can be brought on by the long-term metabolic stress caused by diabetes, regardless of other cardiovascular risk factors.

High Blood Pressure: The heart muscle undergoes structural and functional alterations because of chronic hypertension, most notably left ventricular hypertrophy (LVH), which increases the heart's workload. If left untreated, this adaptation might eventually lead to cardiac failure. One of the main effects of persistently high blood pressure is this condition, which is called hypertensive heart disease. When the diastolic blood pressure is greater than 80 mm Hg or the systolic blood pressure is greater than 120 mm Hg, it is considered hypertension, under the 2017 recommendations of the American Heart Association (AHA) and American College of Cardiology (ACC). According to research, the risk of cardiovascular mortality doubles for every 20 mm Hg rise in systolic or 10 mm Hg increase in diastolic pressure above 115/75 mm Hg (Lewington et al., 2002). The heart responds by growing and thickening to maintain output, but this reduces efficiency over time

Diabetes: Diabetes mellitus (DM) is a significant non-genetic risk factor for the development of cardiomyopathy, specifically a condition known as diabetic cardiomyopathy. This disorder is characterised by myocardial dysfunction without obvious signs of valve disease, hypertension, or coronary artery disease (CAD) (Acar et al., 2011; Li et al., 2020; Nakamura et al., 2022). In individuals with diabetes, chronic hyperglycaemia leads to metabolic disturbances, including oxidative stress, lipotoxicity, and the accumulation of advanced glycation end products. These changes contribute to myocardial inflammation, fibrosis, and left ventricular (LV) remodelling, which impair both diastolic and eventually systolic function of the heart. Early stages are often asymptomatic, but over time, diabetic cardiomyopathy can progress to heart failure (Triposkiadis et al., 2022). Thus, diabetes plays major role in the pathophysiology of cardiomyopathy by causing chronic metabolic and structural changes in the heart, making it a priority for cardiovascular preventive and therapy measures.

Radiations: Radiations are comprehensively used in medical sciences today, like radiation therapy used in cancer treatment, but these radiations welcome various inevitable complications called radiation-induced heart diseases (RIHD). The spectrum of heart diseases includes cardiomyopathy as one of the primary disorders so far caused. Radiation causes damage to endothelial cells, causing them to proliferate, expand, damage, and degenerate, which drastically lowers the capillary count. After the heart was exposed to radiation, von Willebrand factor (vwf) was more readily deposited and released in endothelial cells. The changes in expression led to increasing platelet adhesion and thrombosis in capillaries (Wang et al., 2019)

1.3.2 Genetic Risk Factors

Genetic factors contribute to the development of numerous types of cardiomyopathies, including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic right ventricular cardiomyopathy (ARVCM). These conditions are frequently brought on by mutations in the genes that code for structural proteins in the heart muscle, which result in abnormal electrical activity, cardiac remodelling, and decreased contractility. Mutations in sarcomeric protein genes are prevalent in HCM, including *MYH7* (β-myosin heavy chain) and *MYBPC3* (myosin-binding protein C) (Maron et al., 2003). DCM is usually associated with mutations in genes such as *TTN* (titin), *LMNA* (lamin A/C), and *TNNT2* (troponin T), which alter the mechanical and electrical stability of the myocardium (Hershberger et al., 2013; Kaviarasan et al., 2022). Pathogenic alterations in desmosome genes such as PKP2, DSP, and DSG2 are often detected in ARVC, disrupting cell-to-cell adhesion and promoting cardiac fibrosis and arrhythmia (McNally et al., 2017). The discovery of these genetic variants not only helps with diagnosis and risk classification, but it also has major consequences for familial screening and genetic counselling.

1.3.2.1 β-Myosin Heavy Chain 7 Gene *(MYH7)*

The MYH7 gene, β-myosin heavy chain (β-MHC), a key motor protein for the thick filament of the cardiac sarcomere. This protein is critical for heart muscle contraction because it interacts with actin filaments and hydrolyses ATP to create force. *MYH7* is largely expressed in the ventricular myocardium, where it supports the heart's mechanical needs. Mutations in *MYH7* are significantly linked to inherited

cardiomyopathies, particularly hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy. *MYH7* mutations, primarily missense variations, impair sarcomere contractility in HCM, resulting in myocyte disarray, hypertrophy, and fibrosis (Geisterfer-Lowrance et al., 1990; Richard et al., 2003). These mutations account for 20-30% of all HCM cases and are frequently linked to earlier disease start and more severe clinical characteristics (Seidman and Seidman, 2001; Lee et al., 2018). *MYH7* mutations in DCM decrease sarcomeric force production, causing ventricular dilatation and systolic dysfunction (Hershberger et al., 2013). Furthermore, *MYH7* variations have been associated with uncommon types such as restrictive and arrhythmogenic cardiomyopathies, emphasising the gene's importance in heart health (Walsh et al., 2017; Zhang and Zaho 2025). New variants, like Val431Met, have been found in certain groups, such as Indian cohorts, demonstrating ethnic diversity in genetic vulnerability (Rani et al., 2019). Understanding *MYH7* and its mutations is critical for early detection, genetic counselling, and the development of targeted therapeutics in cardiomyopathy.

1.3.2.2 Angiotensin Converting Enzyme Gene *(ACE)*

The renin-angiotensin-aldosterone system (RAAS) uses the Angiotensin-converting Enzyme (ACE) gene to encode a key enzyme that changes angiotensin I into angiotensin II, a potent vasoconstrictor. *ACE*-II regulates blood pressure, electrolyte balance, and vascular remodelling, all of which have significant effects on cardiovascular health. The insertion/deletion (I/D) polymorphism, which involves a 287-bp ALU sequence in intron 16, is a prominent variant within the ACE gene that modulates circulation and tissue *ACE* levels. The D allele is associated with enhanced *ACE* activity, which may result in raised angiotensin II levels, contributing to myocardial fibrosis, hypertrophy, and ventricular remodelling, particularly under stress situations such as hypertension or ischemia. *ACE* I/D polymorphisms have been studied in dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) to determine their significance in disease susceptibility and severity. Several investigations have found a link between the DD genotype and an increased risk or severity of cardiomyopathy, owing to accelerated fibrotic remodelling and impaired left ventricular function (Cambien et al., 1994; Eijgenraam et al., 2020; Bharti et al., 2024b). Similarly, the D allele has been linked to alcoholic cardiomyopathy, in which people with the DD genotype are more susceptible to alcohol-induced cardiac injury

(Singh et al., 2002). Furthermore, the ACE gene polymorphism may impact therapeutic results in patients treated with *ACE* inhibitors, emphasising its significance in pharmacogenetics and personalised medicine (Cheng et al., 2006).

1.3.2.3 Myosin Binding Protein C3 Gene (*MYBPC3*)

The *MYBPC3* (Myosin Binding Protein C, Cardiac) gene is essential for the structural configuration and function of sarcomere, the fundamental contractile unit of heart muscle. Through modifying actin-myosin interactions and preserving sarcomere integrity, *MYBPC3* plays a role in controlling cardiac muscle contraction. This gene is the most common cause of HCM a disorder marked by thickening of the heart walls, poor relaxation, and a higher chance of sudden cardiac death, is mutations in the *MYBPC3* gene (Carrier et al., 2004; Ananthamohan et al., 2024). These mutations frequently cause shortened proteins or haploinsufficiency, which impairs normal sarcomere activity and causes myocardial fibrosis and hypertrophy (Marston, 2011). *MYBPC3* mutations are occasionally linked to dilated cardiomyopathy (DCM), but less often than they are to HCM. To direct diagnosis and treatment in cases of familial cardiomyopathy, genetic screening for *MYBPC3* mutations is frequently employed. The development of targeted therapeutics for hereditary cardiomyopathies continues to heavily focus on *MYBPC3*, owing to its pivotal role in sarcomere function and heart health (Walsh et al., 2017; Ananthamohan et al., 2023).

1.3.2.4 BAG Cochaperone 3 Gene *(BAG3)*

A multifunctional co-chaperone protein that is crucial for preserving cellular protein homeostasis, particularly in muscle cells and cardiomyocytes, is encoded by the BCL2-associated athanogene 3 (BAG3) gene. In addition to enabling autophagy, especially chaperone-assisted selective autophagy (CASA), which breaks down misfolded proteins and preserves sarcomere integrity, *BAG3* is essential for controlling apoptosis and keeping cytoskeletal structure (Martin, 2021). It strongly collaborates with heat shock proteins, particularly Hsp70, to regulate essential cellular functions, including autophagy, apoptosis, protein quality control, and cytoskeleton maintenance. In the tissues of the heart and skeletal muscles, *BAG3* is extensively expressed and aids in shielding cells from oxidative and mechanical stress. *BAG3* gene mutations have been connected to several illnesses, most notably myofibrillar myopathy and dilated cardiomyopathy (DCM), which are typified by the gradual

weakening of the skeletal muscles or heart (Knezevic et al., 2015). As a possible therapeutic target in degenerative disorders involving protein misfolding and cellular stress, *BAG3* is being thoroughly studied because of its critical function in stress response and cellular protection (Villiard et al., 2011; Tadros et al., 2021).

1.4 Diagnosis

Cardiomyopathy is diagnosed using a combination of imaging methods, electrocardiograms, and invasive procedures to evaluate the anatomy and function of the heart. Many non-invasive techniques are utilised to assess left ventricular dilatation and systolic dysfunction, including cardiac MRI and M-mode and 2D echocardiography with Colour Doppler (Finocchiaro et al., 2020). Although an electrocardiogram (ECG) aids in identifying irregular heartbeats, a chest X-ray is frequently used to detect cardiomegaly, or an enlarged heart. A thorough evaluation may occasionally include endomyocardial biopsy and cardiac catheterisation (Ioannou et al., 2022). Echocardiography is essential for detecting cardiomyopathy since it measures the left ventricular end-diastolic diameter (LVEDD), end-systolic diameter (LVESD), and ejection fraction (LVEF). The formula for calculating LVEF, a crucial measure of heart function, is EF% = (LVEDV - LVESV) / LVEDV × 100, where LVEDV and LVESV stand for the left ventricular volumes at various stages of the cardiac cycle. After controlling for age and body surface area, patients are deemed to have severe systolic dysfunction if their LVEF is less than 45% and their LVEDD is greater than 112% of the expected value. Fractional shortening (FS), a ratio of LVEDV to LVESV, is also examined; values less than 25% signify faulty cardiac function (Goody et al., 2022). Cardiomyopathy patients frequently have aberrant ECG readings, which might include sinus tachycardia, poor R-wave progression, conduction delays, and nonspecific T-wave and ST-segment alterations. Atrioventricular (AV) conduction blockages, atrial fibrillation, or sinus bradycardia can be seen in those with a family history of DCM. As heart failure progresses in more advanced cases, conduction abnormalities and atrial tachyarrhythmias may appear. In DCM patients, ventricular ectopic beats or no sustained ventricular tachycardia are commonly detected by ambulatory ECG monitoring (Trasca et al., 2022). The early detection and successful treatment of disease depend on a comprehensive diagnostic strategy that includes cardiac MRI, ECG assessment, echocardiographic imaging, and catheterisation as required.

1.5 Prevention of Cardiomyopathy

While there is no guaranteed way to prevent cardiomyopathy, there are certain steps you can take to reduce your risk of developing the condition. Here are some strategies that have been shown to be effective:

i. Identify underlying conditions: Certain conditions, such as high blood pressure, diabetes, or obesity, can lead to the development of cardiomyopathy. Managing these conditions through lifestyle changes or medication can help lower the risk of developing cardiomyopathy.

ii. Avoid alcohol and drugs: Excessive alcohol consumption and drug use can damage the heart muscle and increase the risk of cardiomyopathy. Limiting or avoiding these substances can reduce the risk.

iii. Eat a healthy diet: A diet that contains whole grains, fruits, vegetables, lean protein, and healthy fats can help maintain heart health and reduce the risk of cardiomyopathy.

iv. Exercise regularly: Regular exercise can strengthen the heart muscle and reduce the risk of developing cardiomyopathy. However, it's important to talk to a doctor before starting a new exercise program.

v. Get enough sleep: Lack of sleep can contribute to the development of heart disease, including cardiomyopathy. Aim for at least 7-8 hours of sleep per night.

These strategies have been shown to be effective in reducing the risk of developing cardiomyopathy, but it's important to talk to a doctor if you have concerns about your heart health or if you have a family history of heart disease (Maron et al., 2006; Elliott et al., 2014).

1.6 Treatment of Cardiomyopathy

A combination of therapies specific to severity and specific subtypes of cardiomyopathy is used to treat the condition. Pharmacological treatments constitute the primarily use beta-blockers and angiotensin-converting enzyme (ACE) inhibitors improve survival rate. It also reduces hospitalisation in patients with dilated cardiomyopathy (DCM) (Foody et al., 2002). For those who are intolerant of ACE

inhibitors, angiotensin receptor blockers (ARBs) are beneficial alternatives (O'Donovan, 2018). A new myosin inhibitor called Mavacamten, has shown promise in treating hypertrophic cardiomyopathy (HCM) by reducing blockage of the left ventricle's outflow tract and enhancing exercise tolerance (Almansouri et al., 2024). Implanted cardioverter-defibrillators (ICDs) are recommended for high-risk HCM patients to prevent sudden cardiac death. Cardiac resynchronisation therapy (CRT) is another crucial device used for treatment of patients with DCM and severe electrical desynchrony. Heart transplantation is the only proven treatment option for severe cases that are not improving with medication or technological therapy. Novel treatments, including resveratrol, are being researched for their potential cardioprotective advantages in the management of heart failure (Raj et al., 2014). All things considered, treating cardiomyopathy requires a comprehensive, individualised strategy that includes medication, device-based therapy, and, if necessary, surgery.

1.7 Research Area

The proposed study would be carried out in the Jammu region of UT J&K, which is located at 32.73°N 74.87°E and is 300 meters above sea level on average (980 ft). The Shivalik hills, which surround Jammu to the north, east, and southeast, have irregular ridges of modest heights, while the Trikuta Range surrounds it to the northwest.

The current case-control study compares and identifies possible genetic, clinical, and lifestyle-related risk factors linked with cardiomyopathy to better understand the condition's genesis and guide efforts for early identification and prevention. This is accomplished by using curated genetic data from the NCBI database and particular candidate genes that have been linked to cardiomyopathy in the past through genome-wide association studies (GWAS). These genes were chosen for their known functions in the structure, function, and susceptibility of the heart to changes caused by cardiomyopathy. The present investigation determines whether these genetic changes are associated with the disease in Jammu population. This research will contribute to the genetic profiling of the targeted population by identifying the genetic and non-genetic factors and will help to improve public health.

CHAPTER-2
REVIEW OF LITERATURE

2.1 Historical Background

In the 18th century, a physician from London named William Harvey reported the normal circulation of the heart in his classic monograph “De Motu Cordis” (Movement of the Heart) in 1628. But it was still unclear how structural alterations in the heart and the clinical signs of heart failure were related till another researcher, Richard Lower, wrote that when “the parenchyma of the heart suffers from inflammation, abscess or a wound, it may be unable to provide a constant circulation of the blood.” He identified that the heart was quite augmented in patients with heart failure. Since then, efforts have been made to comprehend the actual causes of heart failure. In the late 1800s, heart failure was mainly ascribed to valvular heart disease, which was common at that time. Later in 1891, a physician named Krehl in Leipzig discussed idiopathic cardiac diseases. Followed by Josserand and Galvardin in 1901, who proposed the term “primary myocardial disease” to describe patients with heart failure. An important step was accomplished in 1957 when Dr Wallace Brigden, a specialist in the heart, produced an essay characterising cardiomyopathy as a disease of heart muscle that is not caused by blocked or damaged arteries (Braunwald et al., 2017). The World Health Organisation (WHO) in 1980 characterised cardiomyopathy as "heart muscle diseases of unknown origin" indicating a general lack of etiologic variables that may cause heart failure (Maisch and Bauersachs, 2020). The following WHO categorisation, which was issued in 1995, suggested “diseases of myocardium associated with cardiac dysfunction”.

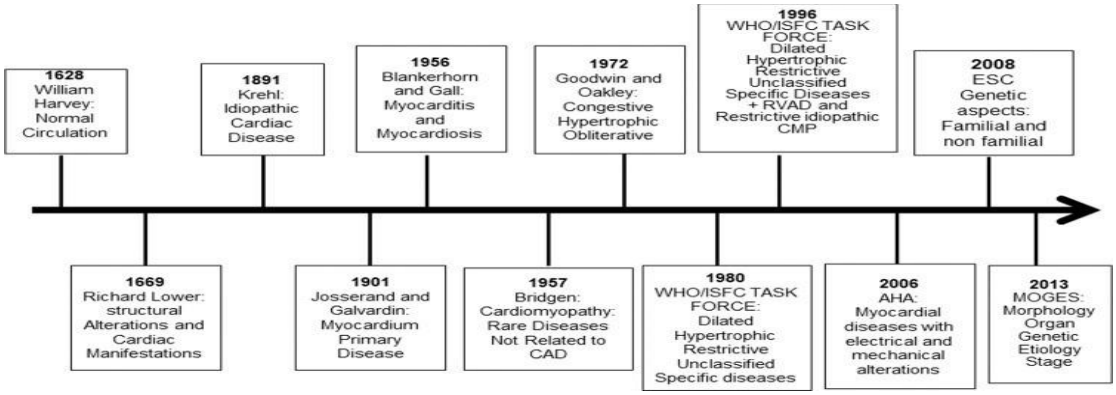


Figure 2.1: Historical Aspects from description of the circulation to Cardiomyopathy classification. AHA, American Heart Association; CAD, Coronary Artery Disease; RVAD, Right Ventricular Arrhythmogenic Dysplasia; CM, Cardiomyopathy; ESC, European Society of Cardiology; WHO/ISFC, World Health Organisation /International Society and Federation of Cardiology.

2.2 Structure of the Heart

Heart is an essential organ that pumps blood throughout the body by regular, involuntary heart muscle contractions, providing all tissues with oxygen and nourishment. With the atria acting as the receiving chambers and the ventricles as the distributing chambers, it consists of comprises four major chambers, divided by a septum: the left atrium, left ventricle, right atrium, and right ventricle in Figure 2.2. Two separate circuits allow blood to flow: the pulmonary circuit involves deoxygenated blood entering the right atrium by the superior vena cava, making its way into the right ventricle via the tricuspid valve, and then being pumped through the pulmonary valve and arteries to the lungs. After being oxygenated, it travels back to the left atrium by the pulmonary veins, entering the left ventricle through the mitral valve and subsequently exits the systemic circuit into the aorta.

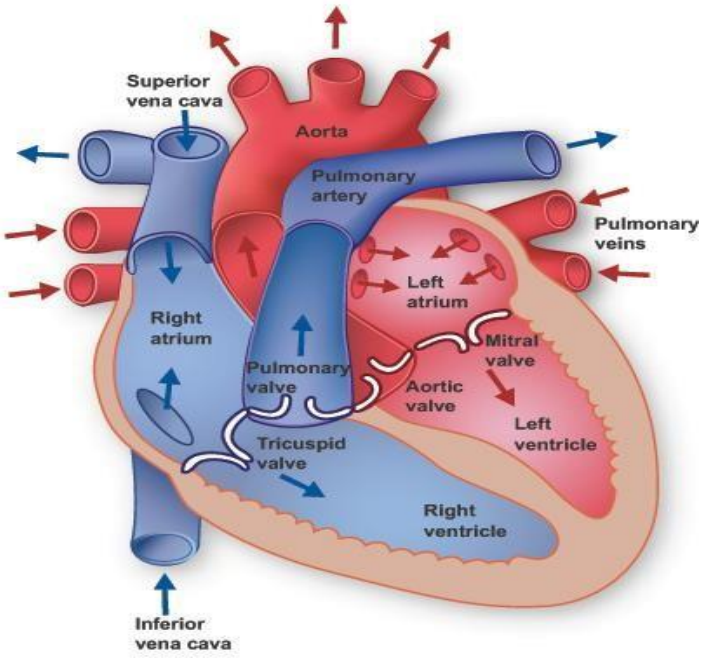


Figure 2.2: Schematic anatomy of the heart

(http://www.texasheart.org/HIC/Anatomy/anatomy2.cfm)

There are three layers in the heart wall: the epicardium, often called the visceral pericardium, forms the inner surface of the pericardial sac. In contrast, the middle layer's myocardium comprises cardiac muscle that propels contraction. Cardiomyocytes are specialised contractile cells that are essential to cardiac function. To maintain practical heart function, these cells cooperate with various cell types,

including endothelial cells, fibroblasts and vascular smooth muscle cells. The extracellular matrix (ECM), which aids in force transmission and contraction regulation, reinforces the synchronized contraction of cardiomyocytes, which is mediated via gap junctions. The mitochondria in cardiomyocytes are closely packed to maintain their constant activity. The sarcomere, a highly ordered structure of repeated units of thick and thin filaments comprising hundreds of proteins, is the primary component of their contractile ability. Any abnormalities in these proteins' expression can interfere with signaling cascades and protein interactions, which may result in heart dysfunction. The heart has amazing adaptive mechanisms to compensate for functional impairments brought on by environmental and genetic causes. Cardiomyopathy results when compensatory reactions are not maintained, causing heart dysfunction (Harvey et al., 2011). Indeed, between 30-50% of instances of cardiomyopathy are linked to hereditary factors (Fatkin et al., 2011).

2.2.1 Cardiac Muscle

The wall of the heart consists of striated involuntary muscle called cardiac muscle. Cells, which are called myocytes. These myocytes are mononuclear and centrally located. The fibres of these myocytes have a diameter ranging from 15-20 μm and a length of 100-200 μm. These muscle cells are branched and connected by intercalated discs, which allow contractile signals to be transmitted between cells (Olivetti et al., 1996). The functional muscle unit is myofibrils; the repeating units called a sarcomere.

2.2.2 Structure of Sarcomere Muscles

Myofibrils are the structural units of cardiac muscle fibers, and they contain contractile elements critical in transforming both chemical and mechanical energy. Sarcomeres, which are repeating units, are joined end-to-end at the Z-disc to form a network of transverse bands that comprise these myofibrils. The spatial relationships of the heart's constituent parts are illustrated in Figure 2.3.

The various functional components of the heart are arranged in a hierarchical framework. Actomyosin cross-bridge proteins are the tiniest; they are nanometer-sized and essential for muscle contraction. The muscular walls of the heart chambers are made up of layers of myocardial tissue that are millimeters thick on a much bigger scale.

The heart may operate well on several levels thanks to the unique configuration (Chein et al., 2008; Sheehy et al., 2012). Sarcomeres are composed of filaments. Whenever a muscle contracts or relaxes, these filaments slide past each other. The cardiac sarcomere, which are 2.2 μm in length, exists typically in a relaxed state. Under the microscope, it appears as alternating dark and light bands. Myosin is the thick filament, which is approximately 1.5 μm in length. They are bonded to other proteins like myosin-binding protein-C (MyBp-C) and myosin light chains. Actin filaments are the thinnest filaments bound to two regulatory proteins, troponin and tropomyosin. These structures attach with Z-line termini, defining the edges of the sarcomere. The microscopic straight appearance of these two filaments causes the overlapping. Under an electron microscope, different zones are present in the sarcomere. The thick filaments appear as an anisotropic A-band region with a dark zone. On the other side, light isotropic I-band represents thin filaments and has a light zone. These thick and thin filaments overlap each other. The intermediate region is called the H-band, where thick filaments are present and thin filaments are absent, and the regions where thick and thin filaments interlock by cross-bridges are referred to as the C-region. During contraction, the both the filaments slide over each other, causing I-band and H-zone to shorten while the A-band remains unchanged. The I-band consists of actin, tropomyosin and troponin T (Martonosi, 2000). The interplay of thick and thin filaments cause muscle contraction as described in the sliding filament theory (Hanson et al., 1953). When actin (thin filaments) bind to myosin (thick filaments), they slide past each other, causing a conformational change, a process powered by ATP hydrolysis (Sherwood et al., 2004). Once the myosin head firmly attaches to actin, ADP is released, restoring the rigour conformation. This continuous binding, sliding, and detachment cycle enables repetitive muscle contractions. Mutations in the sarcomere gene can impair the heart's capacity to contract and relax. The genes that code for vital heart muscle proteins such as Myosin (*MYH7, MYH6*), Myosin-binding protein C (*MYBPC3)*, Actin (ACTC1), Tropomyosin (*TPM1*), Troponin (*TNNT2, TNNI3*), and Myosin light chain 2 (*MYL2*) which can be a contributory factor in Cardiomyopathy along with other causes that result in heart malfunction. It is further exacerbated by structural protein abnormalities that impair the transmission of force required for healthy cardiac function, including Filamin (FLN), Desmin (*DES*), Dystrophin (*DMD*), Sarcoglycan (*SGCD*), Myomesin-1 (*MYOM1*) and Vinculin (VCL),

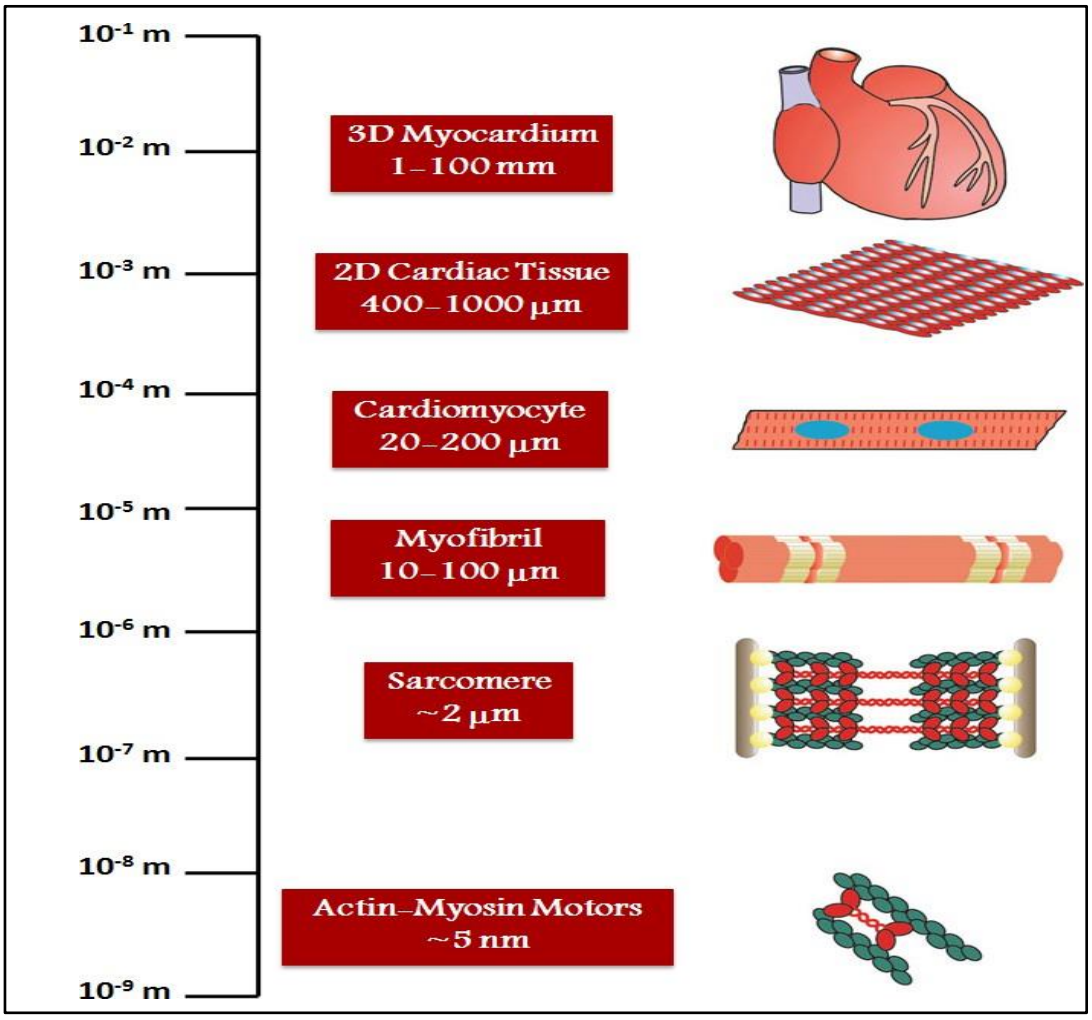


Figure 2.3: Hierarchical Structure of Heart Components (Chein et al., 2008; Sheehy et al., 2012).

2.3 Epigenetics of Heart Failure

Epigenetics refers to a mechanism that modifies gene expression, such as silencing of genes and functions without altering the genome's sequence, thereby leading to heritable changes. Its primary functions include regulating the organization of chromatin and controlling gene expression at various molecular levels to maintain cell identity and regulating cell differentiation, which are crucial for normal development and disease. Epigenetic regulation involves four distinct molecular levels: DNA methylation, modifications to histone proteins following translation, chromatin conformation and remodelling that depends on adenosine triphosphate (ATP), and non-coding RNA. These findings were reported by Wang et al. (2019).

2.4 Cardiomyopathy

It is the primary disorder of heart muscles associated with cardiac dysfunction. It is either acquired or hereditary and is characterised by abnormal thickening or narrowing of cardiac muscle tissue. The heart relies on a complex network of cells to function properly. Cardiomyopathies are a diverse set of disease that frequently result in progressive heart failure and have a high morbidity and death rate (Wexler et al., 2009). Heart muscle disorder called cardiomyopathy makes it more difficult for the heart to pump blood properly. Cardiomyopathy typically results in thickening and enlargement of muscles. It is categorised into two major groups: Primary and secondary cardiomyopathy. Primary cardiomyopathies are predominantly confined to heart muscle, with genetic and non-genetic causes. Secondary cardiomyopathies, on the other hand, are disorders that contribute to harm in myocardial because of systemic or multi-organ disease. Primarily based on morpho-functional state, organ involvement, genetic inheritance, etiology and functional state, cardiomyopathy is broadly classified into the below given following types (Sisakian et al., 2014).

2.4.1 Pathophysiology of Dilated Cardiomyopathy (DCM)

The cardiac muscle condition known as dilated cardiomyopathy (DCM) results in the enlargement of the ventricles and impairs the heart's capacity to contract properly. DCM might eventually cause serious cardiac rhythm issues (arrhythmias) and heart failure. It is characterized by the presence of left ventricular or biventricular dilatation or without congenital heart disease, hypertension, valve disease, or coronary artery disease, systolic dysfunction. A dynamic interplay of genetic, molecular, neurohormonal, and environmental factors results in progressive ventricular dilatation and systolic dysfunction in the etiology of dilated cardiomyopathy (DCM) (Harding et al., 2023). It is a multifactorial cardiac condition marked by left ventricular dilatation and systolic failure. About 40% of cases are genetically linked to mutations in sarcomeric and cytoskeletal proteins, including *TTN, LMNA, FLNC,* and *BAG3*. Recent research has shown the importance of polygenic risk scores in determining susceptibility. Even in those who are genetically susceptible, inflammatory processes such as T-cell-mediated injury and persistent immunological activation lead to cardiac damage and fibrosis. Myocyte hypertrophy, interstitial fibrosis, and myofiber disarray are among the nonspecific histological features that characterize DCM; endomyocardial

biopsy is mainly used to rule out secondary causes. Recent studies also link clonal hematopoiesis and epigenetic changes to the development of illness (Verdonschot et al., 2024). Together, these findings highlight the heterogeneity of DCM and the need for precision medicine approaches in its management (Orphanou et al., 2022).

2.4.2 Pathophysiology of Hypertrophic Cardiomyopathy (HCM)

Hypertrophic cardiomyopathy (HCM), an inherited cardiac disease that cannot be entirely linked to abnormal loading conditions, is characterized by left ventricular hypertrophy. Genetic mutations, anatomical cardiac changes, and functional abnormalities are all part of the complicated etiology of HCM (Gelpi Acevedo et al., 2022). In terms of genetics, hypertrophic cardiomyopathy (HCM) is mostly an autosomal dominant disease cause by abrupt changes in sarcomeric protein genes, including *MYH7* (beta-myosin heavy chain 7 gene) and *MYBPC3* (myosin-binding protein C). This change disrupts the contractile mechanism of the cardiomyocytes, resulting in hypercontractility and inefficient energy usage. This ultimately results in myocyte hypertrophy, disarray, and interstitial fibrosis—all of which are histological traits that are typical of HCM (Spudich, 2019; Gartzonikas et al., 2023). When the myocardium is hypertrophied, it becomes more stiff and less relaxed, which causes defective diastolic function. The left ventricular outflow tract can be blocked by the thickened interventricular septum in many people. This dynamic obstruction exacerbates symptoms and is linked with mitral abnormalities such as mitral regurgitation and systolic anterior motion (SAM) (Varma et al., 2014). Athletes and younger people are more at risk for ventricular arrhythmias and sudden cardiac death due to the arrhythmogenic substrate created by the disordered myocardial architecture and fibrosis. Cardiovascular impairment and increased oxygen demand can cause myocardial ischemia. The pathogenesis of HCM is characterized by genetic abnormalities that result in cardiac hypertrophy, fibrosis, diastolic dysfunction, sarcomere dysfunction, dynamic LVOT blockage, and elevated arrhythmic risk. It is essential to comprehend these pathways to diagnose, classify, and treat individuals with HCM (Marian and Braunwald, 2017).

2.4.3 Pathophysiology of Restrictive Cardiomyopathy (RCM)

Restrictive cardiomyopathy (RCM) is characterized by normal or reduced diastolic and systolic functions. It also causes ventricular wall thickness, and fluctuation in one or both ventricles' volumes (Elliott et al., 2008). While systolic function is usually preserved in RCM, diastolic dysfunction results from increased myocardial stiffness that hinders ventricular filling. This leads to atrial enlargement and increased filling pressures, which are frequently seen as signs of heart failure (Gowda et al., 2022). Accumulation of aberrant chemicals inside the heart causes infiltrative illnesses including cardiac amyloidosis and sarcoidosis, which lead to increased stiffness and impaired diastolic relaxation (Brown et al., 2019). Normal myocardial function is further disrupted by storage diseases, such as hemochromatosis and Fabry disease, which cause metabolic products to accumulate in cardiac tissues (Chintanaphol et al., 2022). Ventricular compliance is also limited by endomyocardial fibrosis, which is characterized by fibrotic thickening of the endocardium. Furthermore, sarcomeric or cytoskeletal protein gene alterations that change myocardial shape and decrease compliance may be the cause of primary RCM. Clinically, patients frequently exhibit peripheral edema and right-sided heart failure. It is introduced by high venous pressures. Bi-atrial enlargement, normal or decreased ventricular sizes, and maintained ejection percentage are typical diagnostic findings. While endomyocardial biopsy may be required to establish a diagnosis, especially in cases of infiltrative or storage-related illness, echocardiography and cardiac MRI are crucial for assessing diastolic dysfunction and ruling out other types of cardiomyopathies (Rapezzi et al., 2022).

2.4.4 Pathophysiology of Arrhythmogenic Cardiomyopathy (ARCM)

Atrial restrictive cardiomyopathy (ARCM), a rare kind of restrictive cardiomyopathy, is characterized by a prevalence of atrial involvement, which hinders ventricular filling due to the increased stiffness of the atrial myocardium. ARCM is characterized by atrial hypertrophy and fibrosis, which impair the atria's ability to contract and store blood. These structural changes may occur alone or in conjunction with ventricular failure. The disturbance of the normal atrial architecture caused by the fibrotic process leads to decreased compliance and lower diastolic filling. This remodeling is often associated with electrophysiological alterations that increase the incidence of atrial arrhythmias, such as atrial fibrillation (AF) (Obremska et al., 2024).

ARCM is caused by several interconnected pathogenic processes that operate at the cellular level and accelerate the illness forward. The fibroblast proliferation and extracellular matrix accumulation are encouraged by chronic inflammation, which results in atrial wall stiffness and fibrosis. Alongside other factors, oxidative stress contributes to cellular damage. It also promotes structural remodeling. Myocardial contractility and relaxation are hampered by disturbances in calcium processing, and energy metabolism is compromised by mitochondrial dysfunction, which exacerbates myocardial dysfunction. The restricted pathophysiology of ARCM is a result of the convergence of several molecular and cellular abnormalities, which affect atrial function (Goette et al., 2024). Genetic mutations play a crucial role in the development of restrictive cardiomyopathies, including ARCM. Genes that produce sarcomeric proteins, such as β-myosin heavy chain (MYH7), troponin T (TNNT2), and troponin I (TNNI3), have been implicated in mutations. Sarcomere dysfunction and increased calcium sensitivity brought on by these mutations may exacerbate fibrosis and diastolic dysfunction (Muchtar et al., 2017; Chintanaphol et al., 2022). In ARCM, the sympathetic nervous system and Renin-Angiotensin Aldosterone System (RAAS) are frequently triggered, which leads to cardiac hypertrophy and fibrosis. This neurohormonal activation worsens diastolic dysfunction and encourages arrhythmogenesis (Kosmala et al., 2024). One of the major morphological and functional changes in ARCM is poor ventricular filling, which results in elevated atrial pressures and pulmonary congestion. Atrial fibrosis and hypertrophy raise the likelihood of AF and other supraventricular arrhythmias. Particularly in the presence of AF, blood stasis in the dilated atria raises the risk of thrombus formation and subsequent embolic events. To manage the course of the disease, early identification of ARCM is essential. The assessment of atrial size, function, and fibrosis requires the use of 2D-echocardiography and cardiac magnetic resonance imaging (CMRI). Assessment of atrial fibrosis can be facilitated by biomarkers like natriuretic peptides and sophisticated imaging methods like late gadolinium enhancement (LGE) in CMRI (Dmour et al., 2021).

2.5 Inheritance of Cardiomyopathy

Cardiomyopathies are an important cause of sudden cardiac death across all ages. Patients with inherited cardiomyopathy are asymptomatic and are diagnosed with family screening.

Autosomal Dominant Inheritance: Most family instances of HCM and DCM are autosomal dominant, meaning that the illness may be brought on by a single copy of the mutant gene and a 50% risk of transmission to a child. The genes *MYH7, MYBPC3*, *LMNA*, and *TTN* are important (Abbas et al., 2024).

X-linked and Autosomal Recessive Inheritance: Rarer forms of cardiomyopathy exhibit autosomal recessive or X-linked inheritance patterns in which both copies of the gene in every single cell have mutations (Morale et al., 2013). It includes certain forms of restrictive cardiomyopathy. Mutations have been connected to genes including TNNI3 and DSP associated with restrictive and arrhythmogenic cardiomyopathies, respectively. The autosomal recessive individual parents carry only one copy of the mutated gene without any symptom of disease (Hershberger et al., 2011).

Mitochondrial Inheritance: Cardiomyopathies, which frequently manifest in children, can result from mutations in mitochondrial DNA. The cardiac muscle cells' ability to produce energy is impacted by these mutations **(**Glavaski et al., 2023**).**

Multifactorial and Polygenic Inheritance: Cardiomyopathies are influenced by environmental variables such as obesity and hypertension, as well as polygenic factors, in addition to single-gene alterations (Yamada and Nomura, 2021).

2.6 Genetic Risk Factors

The genetic aspects of cardiomyopathy have been identified with remarkable progress during the past 25 years (Kim et al., 2021). A significant proportion of patients with cardiomyopathies have heritable diseases, and throughout the last 20 years, the genetic causes of these disorders have been increasingly discovered. Numerous genes have been connected to human hereditary cardiomyopathy because of the substantial genetic variation that characterizes cardiomyopathies, both at the allelic and non-allelic levels (Pascale et al., 2006).

It has been determined that hereditary cardiomyopathies share a number of traits. Initially, distinct phenotypes might be produced by distinct variations within a single gene. HCM, DCM, or RCM phenotypes, can result from mutations in the gene producing the sarcomeric protein cardiac troponin I (TNNI3) (Murphy et al., 2004). In most cases, the same health problem is caused by a unique gene mutation, i.e. a variation causes either DCM or HCM, but not both. Even in cases when the illness gene and allele are identical, there is significant quantitative variation in the specific cardiomyopathy phenotype; this is known as phenotypic heterogeneity.

Genetic Cause: Variants are also known as mutations. There are several sarcomere genes which are involved in cardiomyopathy. The most involved genes are *MYH7, MYH6, MYBPC3* are thick filament motor proteins, *ACTC1* is a thin filament protein, *TNNT2*, and *TNNI3* are troponin complex proteins which maintain calcium sensitivity (Bharti et al., 2024). *TPM1* is a tropomyosin protein which regulates the interaction between actin and myosin (Ho et al., 2015). Apart from these genes, various other genes are involved. These are cytoskeletal protein *DES* and *DMD* (Maggi et al., 2021*)*. *DES* is an intermediate protein that stabilizes the sarcomere and *DMD* connect the cytoskeleton to the extracellular matrix. α-Actinin (*ACTN2)* and *LDB3/ZASP* are z disc protein that's crosslinks the actin filament and helps in signal transduction respectively. *LMNA* is a nuclear protein that acts as a nuclear architecture and is involved in gene regulation (Veltrop et al., 2024). There is certain desmosome proteins Plakophilin-2 (*PKP2*), Desmo plakin (*DSP*), Desmoglein-2 (*DSG2)* which are crucial for intracellular myocardial adhesion (Kostin et al., 2000). *SCN5A* encodes the cardiac sodium channel and control signaling (Remme et al., 2023). Also, sarcomere stability is greatly enhanced by FLNC, a cytoskeletal actin-binding intermediate filament that acts as a linker between membrane proteins and sarcomeres (Bharti et al., 2024). BAG3 (BCL2e Associated Athano Gene-3), RBM20, and TTN are other genes that are linked to sporadic and familial DCM; TTN may account for up to 25% of familial DCM and 18% of sporadic DCM, respectively (Bharti et al., 2024). These genes participate in the production of proteins by cardiomyocytes, which are heart muscle cells. Many of these proteins are found in the sarcomere, which is the structure which allows muscles to contract. Others form up the cytoskeleton, the structural framework of the cell that gives cardiac cells their strength and shape. As evidence of the wider genetic range implicated in cardiomyopathy, mutations have also been found in non-sarcomere genes, such as

those encoding the cardiac muscle LIM Protein *(CLP).* This protein plays a crucial role in the organization of cytoskeletal, gene regulation and signaling pathway (Rangaraju et al 2012). These mutations change the cardiomyocyte function and reduce the ability of these cells to contract (Posafalvi et al., 2013).

Candidate genes are those with recognized biological functions that may play a role in the pathophysiology of a specific disease. To validate their role, the impact of a candidate gene's genetic variations can be assessed using association analysis. Case-control association studies are frequently used to examine the connection between genetic variations and intricate disease states or phenotypes. The allele frequency of genetic markers, such as single-nucleotide polymorphisms (SNPS) or variable number tandem repeats (VNTRS), is compared in such studies between the persons with disease (cases) and healthy individuals (controls) (Tenny et al., 2017). A notable variation in the allele distribution between patients and controls indicates a possible genetic component to either illness protection or susceptibility. It has been determined that several potential genes, either directly or indirectly, play a role in the onset of cardiovascular diseases. This study focuses on the following candidate genes responsible for cardiomyopathy.

2.6.1 Angiotensin Converting Enzyme (ACE) Gene

ACE gene is also known as Dipeptidyl Carboxypeptidase 1 (DCP1) or Kininase II.

Cytogenetic Location of *ACE*:

The ACE gene, situated on 17q23 region of the chromosome's long arm. It spans approximately 26 kb (kilobases) of genomic DNA (Bharti et al., 2025). It has twenty-six exons and twenty-five introns (Figure 2.4 and 2.5).

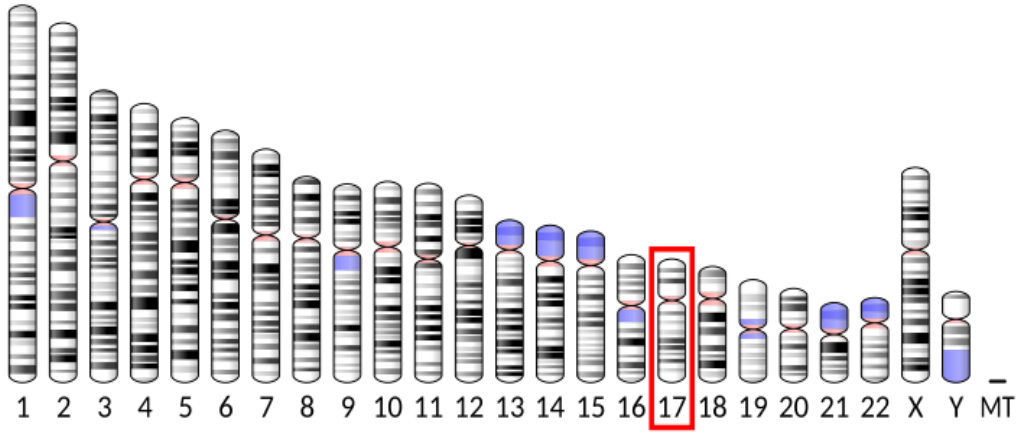


Figure 2.4: Ideogram View of ACE gene (From Ensemble database)

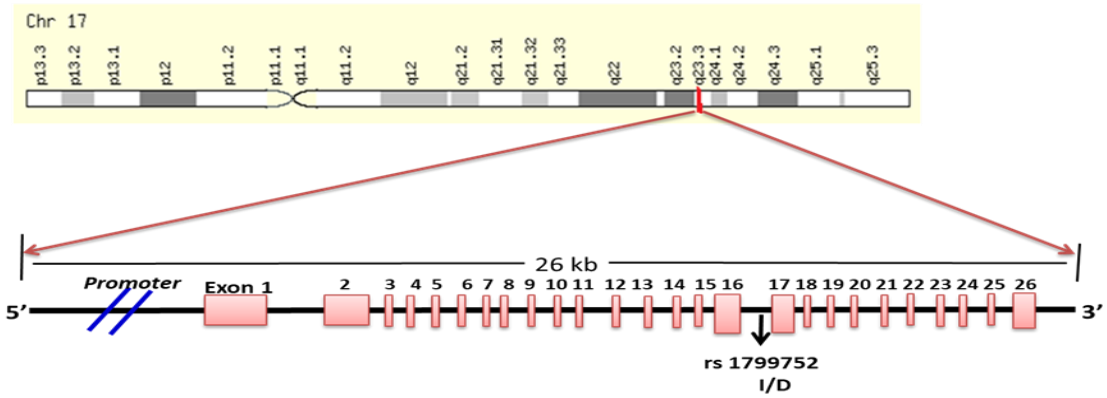


Figure 2.5: Representation of *ACE* gene depicting its cytogenetic location, structure and variant (I/D) (Modified source: www.genecards.org/stat_graphs.shtml).

In terms of molecular location, the ACE gene is located on chromosome 17 between 63,477,060bp to 63,498,379bp. It encodes an angiotensin I-converting enzyme a zinc metalloenzyme dipeptidyl carboxypeptidase. This enzyme is crucial in the renin-angiotensin-aldosterone system (RAAS), which regulates blood pressure and electrolyte balance in the individuals (Bharti et al., 2025). This enzyme converts angiotensin I into angiotensin II, a powerful vasopressor, and degrading vasodilator kinins and also contributes to vascular wall homeostasis (Panjaliya et al., 2013). The ACE gene has two polymorphic variants, one with an insertion (I) and one with a deletion (D) of a 287-bp ALU DNA sequence in intron 16 (ACE gene – GeneCards / ACE protein / ACE antibody) (Bharti et al., 2025). Alu elements are short, interspersed elements (SINES) that account for 5-10% of the human genome and contain a recognition site for the restriction enzyme Alu I (Houck et al., 1979). A typical Alu

element is about 300 bp long and dimeric, with the right half (3' half) being 31 nucleotides longer than the left. The RNA polymerase III promoter (box A and box B) is located on the left arm (Paolella et al., 1983). A short A-rich area separates the Alu dimer's right and left halves. Towards the dimer 3' end, an oligo-d(A) rich tail of roughly 100 bp, a trait shared by all SINES, is present. The homozygous deletion genotype DD of the ACE gene is related to higher levels of ACE enzyme in serum than the homozygous insertion (ID) and heterozygote (ID) genotypes (Bharti et al., 2025). The D allele has been linked to cardiovascular disease since the identification of an angiotensin-converting enzyme insertion/deletion *(I/D)* polymorphism (ACE) gene. Although some studies failed to find such connections, i.e. significant associations of this gene polymorphism with several cardiovascular risk factors have been documented (Uemura et al., 2000). ACE gene deletion polymorphism has been linked to an increased risk of myocardial infarction (Cambien et al., 1994), left ventricular hypertrophy (Schunkert et al., 1994), and coronary artery disease (Nakai et al., 1994). In individuals with cardiomyopathy symptoms can vary from person to person. A person's age, environment, gene mutations, and other genetic modifiers all affect this variance (Perkins et al., 2005).

Structure of *ACE* Gene

The N and C are functional regions of the human ACE gene each have an active zinc ion binding site. There are two different kinds of ACE enzyme found in humans. Both the sperm-specific germinal ACE and the widely distributed somatic ACE are encoded by the same gene but are formed from different promoters during transcription. In comparison with germinal ACE, whose function is mainly unclear, somatic *ACE* possesses two active sites with unique catalytic characteristics. A carboxypeptidase known as *ACE2* has recently been shown to be essential for heart function. Human *ACE* homolog is distinct from ACE in that it eliminates carboxy-terminal hydrophobic or basic amino acids. Even in organisms without a circulatory system or the ability to synthesize angiotensin II, *ACE* homologs have been discovered.

Function of the ACE Gene

The ACE gene encodes for the angiotensin-converting enzyme, a chloride- and zinc-dependent dipeptidyl carboxypeptidase that is widely conserved across mammals.

This enzyme comprises 1,306 amino acids (Soubrier et al., 1988) and is primarily expressed in vascular endothelial cells and epithelial cells of various organs, including the kidneys, heart, and blood vessels (Marre et al., 1996).

ACE gene plays a crucial function in the renin-angiotensin-aldosterone system (RAAS) (Ahluwalia et al., 2009), where it regulates blood pressure (BP) and fluid-salt homeostasis. The enzyme catalyzes the cleavage of a C-terminal dipeptide (His-Leu) from angiotensin I (Ang-I), converting it into angiotensin II (Ang-II), a strong vasoconstrictor. Additionally, *ACE* inactivates bradykinin, a vasodilatory peptide, through its proteolytic activity (Eisenmann et al., 2009). Pharmacological the suppression of *ACE* has been found to lower cardiovascular disease risk, notably for individuals with diabetes mellitus (T2DM) (Marre et al., 1997). *ACE* also plays role in coronary artery disease and stroke (Sayed et al., 2006).

ACE (*I/D* Polymorphism)

The ACE gene has more than 160 polymorphisms known to date. The presence (insertion, II) and absence (deletion, DD) of a 287-base pair (bp) Alu element sequence in intron 16 characterizes the Insertion/Deletion (I/D) variation, one of the most researched polymorphisms (Sayed-Tabatabaei et al., 2006). This polymorphism significantly influences *ACE* enzyme activity, accounting for approximately 50% of the circulating and tissue *ACE* levels variability. The strongest *ACE* activity is linked with DD genotype, whereas the II genotype corresponds to the lowest *ACE* activity (Sakuma et al., 2004). The D allele has been linked to increased *ACE* levels and has been frequently observed in patients with cardiomyopathy. Although the I/D polymorphism is locating in a non-coding region (particularly an intron) of the *ACE* gene, multiple researchers have discovered that the D allele is associated with higher *ACE* activity in blood. Numerous diseases have been related to *ACE* I/D polymorphism by research done worldwide. including coronary heart disease, stroke, hypertension, and diabetes mellitus (Zee et al., 1999; Kennon et al., 1999; Obineche et al., 2001; Gesang et al., 2002). However, inconsistent findings regarding the relationship between *ACE* polymorphism and illness have been published (Moleda et al., 2007; Taal et al., 2000). Furthermore, some studies have found inter-ethnic differences in the frequency of allelic variants of the ACE genes (Barley et al., 1994; Saha et al., 1996). Aldosterone-stimulating peptide angiotensin II has a strong and direct vasopressor effect on the

peripheral vasculature. It is essential for electrolyte and circulatory balance. The dipeptidyl carboxypeptidase- *ACE* catalyzes its conversion from its precursor, angiotensin I (Koh et al., 2003).

The correlation between sickness and the ACE gene I/D polymorphism was investigated in a case control study involving 80 HCM patients and 88 of their unaffected relatives. The D allele was much more prevalent in HCM patients (0.42) compared to their unaffected relatives (0.35) and show a significant p-value of less than 0.05. Also, the stratified analysis showed that the D allele was more prevalent in people with sporadic HCM (SHCM) than in those with familial HCM (FHCM), which once more achieved statistical significance ($p < 0.05$). Using the calculated odds ratios, this association was further corroborated by an overall OR of 1.98 for HCM, 1.46 for FHCM, and 2.97 for SHCM. These data indicate a greater genetic vulnerability linked with the D allele in sporadic occurrences of HCM, suggesting that the starring role of *ACE* in the development of cardiac hypertrophy, particularly in non-familial forms of the condition (Yoneya et al., 1995).

Higher *ACE* levels in the blood are associated with the DD genotype linked to both idiopathic dilated and ischemic cardiomyopathy. This study examined the role of *ACE* (I/D) polymorphism in tachycardia-mediated cardiomyopathy. In it, 20 individuals with disease (Group A) compared to 20 patients with tachycardia. However, it preserved ejection fraction (Group B) and 24 healthy controls (Group C). After 30 months, Group A showed significant improvement in ejection fraction ($p<0.001$). The DD genotype was more frequent in Group A than in Groups B and C ($p<0.035$ and $p<0.009$, respectively). Findings indicate *ACE* I/D polymorphism may contribute to cardiomyopathy secondary to tachycardia (Deshmukh et al.,2000)

The effect of the ACE *I/D* polymorphism on gene expression and left ventricular function in ischemic disease patients after coronary artery bypass grafting (CABG) was observed. LV function was assessed by ventriculography prior to surgery, and 50 participants were genotyped using PCR. The ACE gene's expression in heart tissue was evaluated in 46 people using quantitative RT-PCR. Because left ventricular ACE expression varied significantly with ACE genotype and was associated with left ventricular function, the results suggested that this polymorphism may contribute to cardiac remodelling in ischemic heart disease (Davis et al., 2000).

The morphological abnormalities in intramural coronary arterioles (small vessel disease) likely underlie microvascular dysfunction, leading to impaired vasodilation and reduced myocardial blood flow during stress (hypoperfusion) (Maron, 2002).

There is a link between *ACE* polymorphism and cardiomyopathy in 174 patients and 164 healthy individuals. The research found that the *ACE* genotype (DD) and allele (D) were more common in patients with hypertrophic (HCM) and dilated cardiomyopathy (DCM), increasing the risk of these conditions. Cardiomyopathy was more frequent in males. HCM patients with ID and DD genotypes had thicker heart walls, but the difference was insignificant. The ID genotype was linked to lower heart function (LVEF) in DCM patients. These findings suggest that the *ACE* D allele may contribute to the development of HCM and DCM (Rai et al., 2007). Additionally, high dietary salt intake was significantly linked to increased obesity risk, especially among those with the DD and ID genotypes (Gupta et al., 2009).

The relationship between the ACE gene polymorphism and hypertrophic cardiomyopathy (HCM) through a systematic review and meta-analysis of 15 case-control studies had been analyzed. The analysis included 2972 participants (1047 HCM cases and 1925 controls). Pooled odds ratios indicated that allele D was a risk factor for HCM across all genetic models. These results suggest that this polymorphism is likely a genetic risk for HCM (Yuan et al., 2017).

Peripartum cardiomyopathy (PPCM) studies revealed that PPCM patients had considerably higher levels of the DD genotype and the D allele than controls. Moreover, systolic performance was poorer in those with the DD genotype, indicating a possible involvement of the *ACE* polymorphism in the aetiology and severity of PPCM (Yaqoob et al., 2018).

In a recent study, Sabir et al. (2019) examined the association between *ACE I/D* polymorphism, serum ACE levels, and obesity risk in 267 adult Saudi volunteers. They discovered that the genotype DD was associated with higher serum ACE enzyme activity and a higher prevalence of obesity than the other genotypes.

The relationship between the risk of cardiomyopathy in the Jammu population and the *ACE I/D* polymorphism. A substantial association was found in a case-control study, indicating genetic propensity. A meta-analysis of 34 relevant research was

carried out to validate these results, and it verified the correlation with both hypertrophic and dilated cardiomyopathy. The findings highlight how crucial genetic research is to comprehend the genesis of cardiomyopathy (Bharti et al., 2025).

The effect of the *ACE I/D* polymorphism on cardiac function and obesity in heart failure patients was evaluated in cross-sectional research. The results of the study showed that a greater incidence of dilated were significantly associated with carriers of the D allele (DD and ID genotypes). Nevertheless, there was no discernible correlation between polymorphism and obesity measurements (Vale et al., 2025).

2.6.2 Myosin Heavy Chain 7 (MYH7) Gene

Cytogenetic Location of *MYH7*

The cytogenetic location of the MYH7 gene is 14q11.2. This gene is present in tandem on chromosome 14 (Figure 2.6 and 2.7). On the band, it starts right from the 23,412,740 bp and ends at the 23,435,660 bp. The total exon count for this gene is 41.

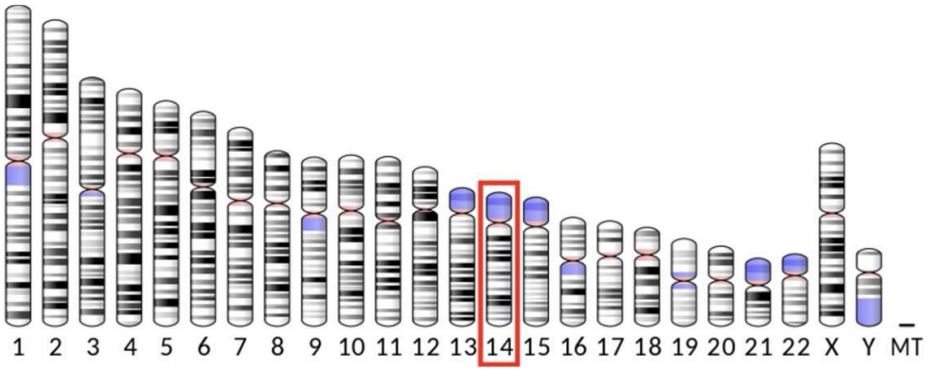


Figure 2.6: Ideogram view of MYH7 gene (From Ensemble database)

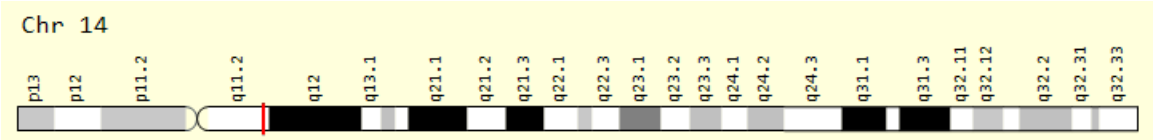


Figure 2.7: Cytogenetic location of MYH7 gene [From Gene cards].

Structure of *MYH7*: Muscle myosin is a protein which encompasses 23 kb of genomic DNA. This gene is encoded by the cardiac myosin's beta (fast) heavy chain subunit. It is primarily derived from skeletal muscle tissues as well as the typical human ventricle. The contractile velocity of cardiac muscle is influenced by the quantity of protein alpha.

It is the fast heavy subunit of cardiac myosin. Myosin storage myopathy, hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are linked to mutations in this MYH7 gene. There are over 200 identified modifications in this gene that consequence in DCM and HCM (Loiben et al., 2023).

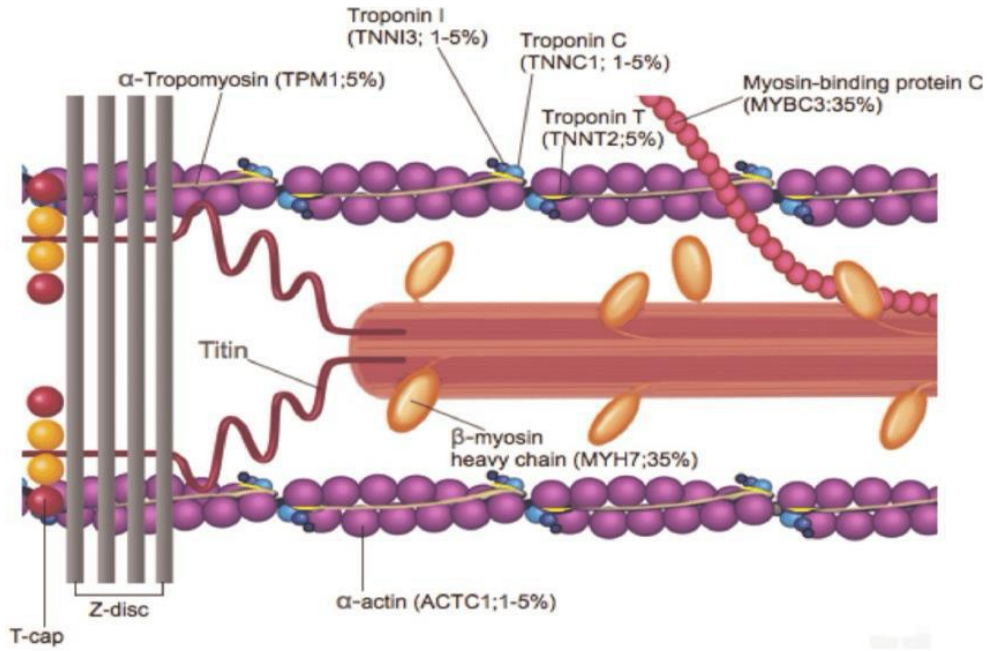


Figure 2.8: Image depicting location of MYH7 genes in the sarcomere (Maron et al., 2013)

The major sarcomere gene provides instructions for making a protein known as the beta myosin heavy chain (β-MHC) in Figure 2.8. The heavy chain of β-myosin in cardiac and skeletal muscle cells creates a bigger protein known as type II myosin. Each type II myosin protein consists of two heavy chains and two pairs of regulatory light chains. Type II myosin generates the mechanical force muscles which helps in contraction (Gao et al., 2023).

Functions of MYH7 Gene

The myosin binding heavy chain gene in humans encodes the beta-myosin heavy chain, a fundamental protein in the cardiac and slow-twitch skeletal muscles. Here are the key functions of the MYH7 gene

1. **Muscle Contraction:** Cardiac Muscle: The beta-myosin heavy chain is a major component of the thick filaments in cardiac muscle sarcomeres. It plays a

critical role in the heart's contractile process by interacting with actin filaments, generating the force required for heartbeat conduction. Skeletal Muscle: In slow-twitch skeletal muscles, the *MYH7* gene product contributes to sustained, long-duration contractions important for posture and endurance activities.

2. **Energy Efficiency:** The beta-myosin heavy chain has a lower ATPase activity than fast-twitch myosin heavy chains. This lower ATPase activity benefits energy efficiency, allowing the heart and slow-twitch muscles to contract repeatedly without excessive energy consumption.

3. **Regulation of Contractile Properties:** The *MYH7* gene encodes a protein that influences the velocity of muscle contraction and the mechanical properties of muscle fibres. In the heart, this regulation is essential for maintaining proper cardiac output and adapting to varying physiological demands.

4. **Structural Integrity of Sarcomeres:** The beta-myosin heavy chain is essential for sarcomeres' structural integrity and proper assembly. This role is crucial for the cardiac and skeletal muscles' overall stability and function.

5. **Role in Development and Adaptation:** The expression of *MYH7* can change during development and in response to physiological or pathological stimuli. For example, in response to endurance training or heart disease, *MYH7* expression can be upregulated to enhance the contractile efficiency and adaptability of the muscle.

6. **Implications in Disease:** Cardiomyopathies: *MYH7* is linked to several forms of cardiomyopathy, including dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM) and restrictive cardiomyopathy (RCM). These conditions often result from structural and functional alterations in the myosin protein, impairing muscle contraction and leading to heart dysfunction.

Congenital Myopathies: Some mutations in *MYH7* can also cause congenital myopathies, a group of genetic disorders characterised by muscle weakness and structural abnormalities in muscle fibres. Overall, *MYH7* plays a vital role in the function and health of both cardiac and slow-twitch skeletal muscles, influencing their

contractile properties, energy efficiency, structural integrity, and adaptability (Walter et al., 2008).

MYH7 Polymorphism

MYH7 gene polymorphism refers to variations in the DNA sequence of this gene that are relatively common within the population. These variations can involve single nucleotides, substitutions, SNPS, insertions, deletions, or other genetic changes. The rs397516208 polymorphism in the *MYH7* gene is a specific single-nucleotide variant (SNV) identified in genetic studies. This variant represents a change in the DNA sequence of the *MYH7* gene at a specific position, i.e., substitution from G>A (C4276G>A). The amino acid change present is Glu1426 Lysine (p.Glu1426lys). The variant type is a protein called E1426K, which is an SNV (single-nucleotide variation). Located in exon 31 of *MYH7* within its rod domain. At codon 1426 of the *MYH7* protein the acidic and polar glutamic acid is substituted with lysine, which is basic and polar (Siewertsen et al., 1990).

The sequence variation in the gene coding for the beta-myosin heavy chain was observed. Their study analysed variations in the 5808-bp MYH7 gene among 25 individuals without familial hypertrophic cardiomyopathy (FHC). Six SNPS were identified, none of which altered the sequence of amino acids. Nucleotide diversity was low compared to other genes. Analysis suggests *MYH7* has evolved slowly, with intense selective pressure against mutations causing cardiomyopathy (Freeman et al., 2001).

The Beta myosin heavy chain gene is a major target of mutation screening in dilated cardiomyopathy. In 96 individual cases (54 familial and 42 sporadic), each exon encoding the beta myosin heavy chain (MYH7 gene) was examined for mutations using SSCP and sequencing. The study discovered heterozygous mutations in the MYH7 gene (either in the head or tail domain of the protein) in seven cases. The results showed that *MYH7* is the most prevalent gene mutation in our familial dilated cardiomyopathy (FDCM) group (about 10%), and carriers of this mutation showed delayed onset. In none of the patients did DCM correlate with skeletal myopathy or conduction problems (Villard et al., 2005).

The genetic variants in the β-MYH7 gene are linked with HCM and DCM. To find single-nucleotide polymorphisms (SNPs) and mutations, they used PCR and

single-stranded conformation polymorphism (SSCP) analysis on samples from 95 HCM patients, 97 DCM patients, and 100 controls. Both HCM and DCM patients exhibited typical genetic mutations in exons 19, 12, and 7 of the MYH7 gene. They concluded that these variations may contribute to reduce energy compromising, dosage effects of mutant proteins, or environmental and modifier gene influences, potentially cause disease (Tanjore et al., 2010).

The Japanese individuals suffering from hypertrophic cardiomyopathy were subjected to genetic screening and double mutation. A study was investigating the genetic basis of hypertrophic cardiomyopathy (HCM) in Japanese patients, focusing on mutations in sarcomere genes. Among 93 patients, 14 mutations were found, with two patients having double mutations (P106S/R869C and R945fs/E1049D) in the *MYBPC3* and *MYH7* genes in HCM (Kubo et al., 2011).

The low occurrence of *MYH7* mutations in familial hypertrophic cardiomyopathy (FHC) patients in India. They analysed clinical and molecular data from FHC patients, identifying *MYH7* mutations using PCR-DNA sequencing. Among 55 patient samples, only nineteen showed mutations, with five having *MYH7* mutations and two exhibiting double heterozygosity. The mutations were primarily missense, affecting conserved amino acid residues and likely altering the protein structure (Bashyam et al., 2012).

When the whole MYH7 gene was sequenced in 60 patients with hypertrophic cardiomyopathy (HCM) who did not have known sarcomere mutations, several rare intronic and promoter variants were discovered, but no exon deletions were discovered. Although a potential splicing-affecting intronic variant was identified, its incidence was similar in patients and controls. Patients had a higher prevalence of rare promoter alterations, some of which were in transcription factor binding areas and may impact gene expression. One rare 3' UTR variant was found, and it most likely has no functional implications. Even though non-coding MYH7 mutations are uncommon in HCM, promoter alterations may generally raise the risk of disease (Coto et al., 2012).

Genetic heterogeneity in hypertrophic cardiomyopathy was revealed by high-throughput sequencing (HTS). They examined the clinical implications of high-throughput sequencing (HTS) for hypertrophic cardiomyopathy (HCM). They analysed

223 patients using targeted HTS, identifying rare variants in 41 cardiovascular genes. Results revealed an abundance of unusual changes in sarcomeric genes, i.e. *MYH7, MYBPC3, TNNI3,* and *TNNT2,* with probabilities of pathogenicity ranging from 57% to near certainty. Additionally, they found novel variants in desmosomes and ion channel genes. This study sheds light on the prevalence of gene variants in HCM patients using HTS, highlighting the challenge of interpreting variants of unknown significance (Lopes et al., 2013).

Genetic variations in the beta MYH7 gene among Venezuelan patients with hypertrophic cardiomyopathy (HCM) were observed. They examined 58 HCM patients and 106 control subjects, analysing coding regions and exon-intron junctions using genomic DNA isolation, polymerase chain reaction, and sequence analysis. Eight known polymorphic variants and two intronic variations were identified, but no missense or pathological mutations were found in the MYH7 gene among the HCM patients (Rodriguez et al., 2014).

Whole-exome sequencing was done to determine mutation of *MYH7* in a Chinese family with left ventricular noncompaction. They stated that left ventricular noncompaction (LVNC) is a genetic heart condition caused by embryogenesis-related developmental issues. This study focused on a Chinese family with LVNC, using whole-exome sequencing to identify potential causal mutations. They discovered a novel mutation (c.C1492G) in the MYH7 gene, likely responsible for LVNC within the family. This mutation was found to be statistically significant and predicted to be deleterious. The study reaffirmed *MYH7's* role as a pathogenic gene for LVNC (Yang et al., 2015).

Research investigated the detection of SNPs in the cardiomyopathy-associated MYH7 gene using an electrochemical primer extension technique. To perform the extension, researchers employed an electrochemical setup with specifically labelled ddNTPs. The efficacy of the procedure was validated by the successful integration of all four forms of ddNTPs into immobilised primers. This strategy is a promising first step toward developing a system that is both economical and effective for detecting genetic variants linked to illness (Debela et al., 2016).

A novel mutation that contributes independently to Left ventricular hypertrophy in a family where a *MYH7* mutation is known. They reported that a 62-year-old male with severe left ventricular hypertrophy (LVH) had a novel mutation in the HRAS gene, not the usual sarcomere gene mutation seen in his family. This mutation, also found in his daughter with mild LVH and intellectual disability, activates the RASMAPK pathway, causing LVH. This finding underscores the importance of RAS signalling in LVH and the challenge of diagnosing RASopathies, especially when symptoms overlap with other conditions like hypertrophic cardiomyopathy (Sana et al., 2016).

The examination of the potential correlation between hypertrophic cardiomyopathy and MYH7 sarcomeric gene polymorphism and incidence of atrial fibrillation. According to the study, 1040 adult HCM patients with either *MYH7, MYBPC3,* or thin filament genes that were likely pathogenic or had pathogenic variation were included, even if they did not have baseline atrial fibrillation. Atrial fibrillation (AF) was most common in individuals with potentially pathogenic or pathogenic mutations in *MYH7*, according to research that controlled for sex, age, proband status, left atrial size, maximum wall thickness, and peak pressure gradient (Lee et al., 2018).

The MYH7 gene's promoter region includes a conserved G-rich 23-base sequence (HM23), which has been connected to familial cardiomyopathy. Researchers demonstrated that this sequence may form G-quadruplex structures with varying strand counts (two, three, or four) but the same parallel orientation using methods such as gel electrophoresis, UV melting, and circular dichroism. They discovered that the formation of a distinct three-stranded G-quadruplex requires a continuous length of five guanines (G5). The formation of the three-stranded structure is inhibited by mutations that break this stretch. These results demonstrate how base variations may impact DNA structure, which in turn may impact illness and gene control (Singh et al., 2018).

A study that addressed *MYH7* examined the clinical consequences of sarcomere gene mutations in 7,675 individuals with hypertrophic cardiomyopathy (HCM). The results demonstrated that ventricular arrhythmias, heart transplantation, and cardiac conduction abnormalities were more common in individuals with *MYH7* mutations than in those without. Furthermore, *MYH7* mutation carriers had a more severe form of HCM and developed the condition earlier in life, according to genotype-phenotype analysis.

These findings demonstrate how *MYH7* mutations significantly affect the severity and clinical course of HCM (Sedaghat et al., 2018).

Genetic analysis of monoallelic double *MYH7* mutations found in families in China with hypertrophic cardiomyopathy (HCM), focusing on the myosin heavy chain (MYH7) gene. Using targeted exome sequencing, they analysed data from 387 HCM probands and their families from 2013 to 2017. They found compound heterozygous mutations in 4 probands and monoallelic double mutations in a single proband. Family members with monoallelic double mutations showed similar mutations and cardiac abnormalities. Echocardiography revealed differences between compound heterozygous and monoallelic double mutation probands. Three-dimensional modelling showed structural changes in mutated proteins. This study identified novel HCM-causing *MYH7* mutations and reported a rare HCM family with monoallelic double mutations (Wang et al., 2019).

In a study, the examination of allele-specific knockdown of *MYH7* was done by using antisense oligonucleotides. They explored using locked nucleic acid (LNA)-modified antisense oligonucleotides (ASOS) to selectively target mutations in the myosin heavy chain 7 (*MYH7*) gene, common in hypertrophic cardiomyopathy. In *MYH7,* three SNPs identified and designed ASOS to target specific alleles, successfully demonstrating allele-selective knockdown in mouse models, indicating a promising therapeutic approach for cardiac pathology (Anderson et al., 2020).

The research investigation of new *β-MYH7* gene variants in individuals from India. They sequenced the *β-MYH7* gene in 167 ethnically matched healthy controls and 137 Indian DCM patients to find the prevalence of mutations and their correlations. For the first time, seven mutations (8.0%) of the 27 variants found in the results were found solely in Indian DCM patients. A frameshift mutation (1.5%), two splice-site mutations (3.6%), and four missense mutations (2.9%) were among them (Rani et al., 2021).

The impact of female sexuality on variations in sarcomeres and medical outcomes: Hypertrophic Cardiomyopathy (HCM). They found that despite similar genetic testing rates, women with HCM were older at diagnosis and more likely to have pathogenic sarcomere variants compared to men. Although implantable cardioverter

defibrillator usage and ventricular arrhythmia did not differ by sex, women had higher all-cause mortality rates. Regardless of the genotype, women with HCM faced a greater risk of mortality and severe heart failure symptoms, indicating a potential sex-related influence on long-term myocardial performance in HCM. Further research is needed to explore this effect (Lakdawala et al., 2021).

The examination of age and gender differences in the genetics of cardiomyopathy in 1,397 patients in Ontario, UK was conducted. Among them, paediatric and adult cases were 471 and 926, respectively. The number of *MYH7* mutations in hypertrophic cardiomyopathy was higher in pediatric patients than in adult females. But in adults, OBSCN was a top-mutated gene due to genetic differences. It clarifies the differences in cardiomyopathy penetrance linked to age and sex. The prevalence of mutations in *MYH7* was greater in individuals with pediatric cardiomyopathy and more likely to be genotype-positive than in adults (Akinrinade et al., 2023).

An autosomal dominant type of dilated cardiomyopathy may be brought on by a new heterozygous missense mutation in *MYH7*. The study examined a proband from an Iranian family with myosin storage myopathy (MSM), characterised by proximal muscle weakness and dilated cardiomyopathy. Whole-exome sequencing identified a novel heterozygous missense variant, predicted to be deleterious. The variant was found in the proband and their children, confirmed by Sanger sequencing (Naderi et al., 2023).

The investigation of how heightened calcium sensitivity in muscles of patients exhibit disrupted calcium homeostasis and diastolic impairment, resulting in cardiac dysfunction with *MYH7* mutation. By comparing mutant cardiomyocytes to control cells from induced pluripotent stem cells, they observed hypertrophy, unusual calcium handling, and intensified myofilament calcium sensitivity in mutant cells. Treatment with mavacamten, which inhibits calcium sensitivity, improved hypertrophy. This study underscores the role of heightened myofilament calcium sensitivity as a key mechanism in HCM, suggesting potential therapeutic approaches (Guo et al., 2024).

The examination of clinical phenotypic traits in hypertrophic myocardiopathy patients with the *MYH7-R143Q* mutation was done. In this study, 1023 unrelated HCM patients were gathered, those who carried *MYH7-R143Q* were subjected to Sanger

sequencing, and clinical data were examined. *MYH7-R143Q* had a detection rate of 2.54 percent (26/1023). Patients with *MYH7-R143Q*-carrying HCM are often diagnosed between the ages of 31 and 40, with mild fibrosis and hypertrophy (Zhang et al., 2024).

2.6.3 Myosin Binding Protein C3 (MYBPC3) Gene

The sarcomere represents the basic structural and fundamental unit of contractility of striated muscles. The Z-discs surround the M-disc, which forms the centre of a symmetrical unit. Z-disc and M-disc provide anchorage for thin and thick elastic filaments (Hanson and Huxley, 1953). The enormous protein titin, which covers half of the sarcomere, makes up the elastic filament. The regulatory proteins tropomyosin and troponin, as well as filamentous actin, are the primary components of the thin filament. Myosin, a motor protein, and Myosin Binding Protein C (*MYBPC3*), a regulatory protein, link the thick filament to the thin filament (Huxley and Hanson, 1954). The sarcomere shortens, myocytes constrict, and ultimately the entire muscle contracts due to myosin's interaction with actin under the influence of Ca^{2+}. The sarcomere arrives at the nucleus and extracellular space via the cytoskeleton. It is made up of several highly organised proteins in addition to proteins of thin and thick filaments (Huxley and Nieder Gerke, 1954). The sarcomere is also linked to numerous signalling pathways by a variety of related proteins. Therefore, the efficient operation of each sarcomere within each cardiomyocyte of the heart muscle is crucial for the contractile function of the heart and is based on the extremely well-balanced interaction of sarcomere proteins and of the sarcomeres themselves (Bobyleva et al., 2021).

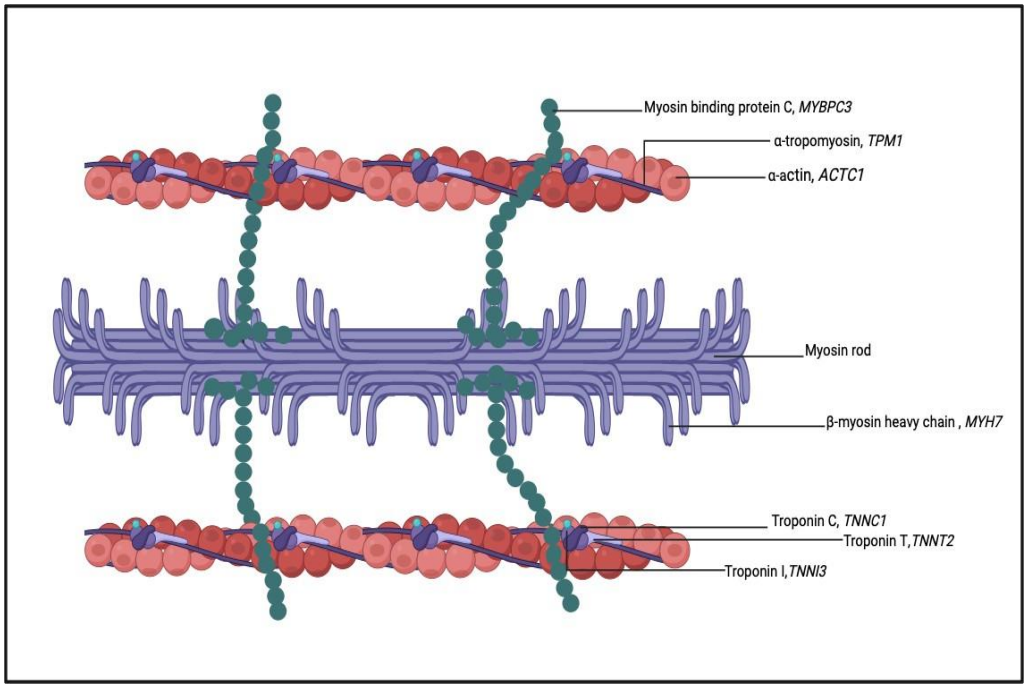


Figure 2.9: Image depicting the location of MYBPC3 gene in the sarcomere (Maron et al., 2013).

Cytogenetic Location of *MYBPC3*

The MYBPC3 gene is positioned on chromosome 11p11.2. It codes for cardiac myosin-binding protein C (cMyBP-C) (Carrier et al., 2015). Twenty years ago, it was identified on chromosome 11 by the Schwartz group. It was the ideal gene for this locus because it is a part of the sarcomere that interacts with titin, myosin, actin, (Luther et al., 2011).

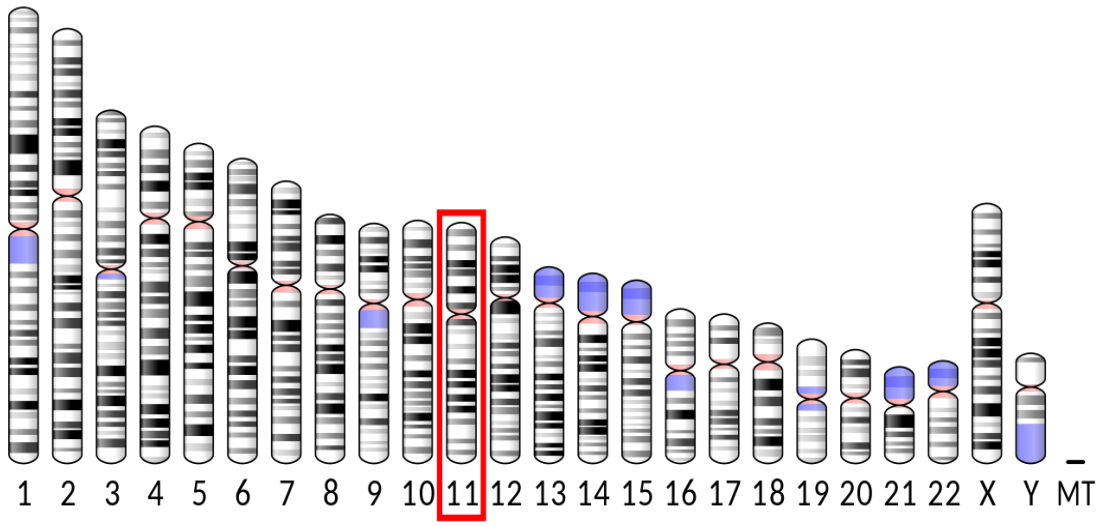


Figure 2.10: Ideogram view of MYBPC3 gene (From Ensemble database)

Thus, *MYBPC3* was the fourth HCM gene in chronological order, after *MYH7*, which encodes the myosin heavy chain, *TNNT2* and *TPM1*, which, respectively, encode cardiac troponin T and tropomyosin.

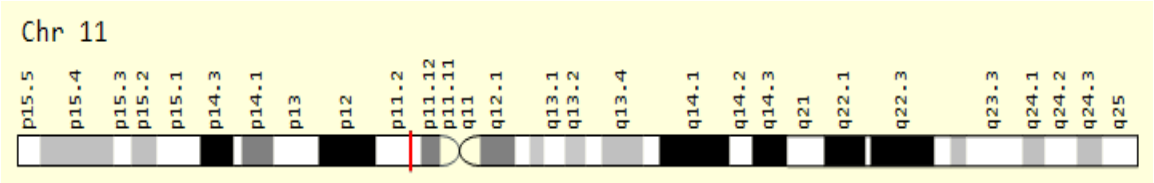


Figure 2.11: Representing cytogenetic location of MYBPC3 gene; [From Gene Cards].

MYBPC3 abnormalities are associated with hereditary cardiomyopathy (Bhola et al., 2018). Along with myosin and titin, early in mammalian heart development, cMyBP-C is expressed. The expression of the skeletal MyBp-C isoforms occurs later in the development of the skeletal muscle than that of myosin and titin, with skeletal MyBp-C expression occurring before rapid skeletal MyBp-C expression. There have also been reports of MyBP-C in an embryonic form that develop alongside titin and myosin. This early form of MyBP-C in skeletal muscles is thought to be the cardiac isoform in both chicken and axolotl (Fougerousse et al., 1998).

Structure of *MYBPC3*

Human MYBPC3 gene structure and sequencing were established in 1997. It has 35 exons, including 34 coding exons, 1274 amino acids, a 140-kDa protein and is larger than 21 kbp (Carrier et al., 2015). A multi-modular structural protein called MyBP-C makes up the sarcomere (Hanson and Huxley, 1953). It embellishes the C-zone of the A band, generating doublet-appearing transverse stripes in the cross-bridge carrying region that are spaced 43 nm apart from one another. The MYBPC1 gene shows the slow skeletal isoform of MyBp-C, whereas the MYBPC2 gene on chromosome 19q33.3 encodes the fast skeletal isoform. Adult human muscle contains the cardiac isoform of MyBp-C, or cMyBP-C. The cardiac isoform is uniquely expressed in the developing hearts of both humans and mice (Fougerousse et al., 1998). Twelve domains make up the cardiac isoform of MyBP-C, including three fibronectin type-III (FN3) domains, eight immunoglobulin (IgC2)-like domains, and one phosphorylation (M) domain (Tudurachi et al., 2023).

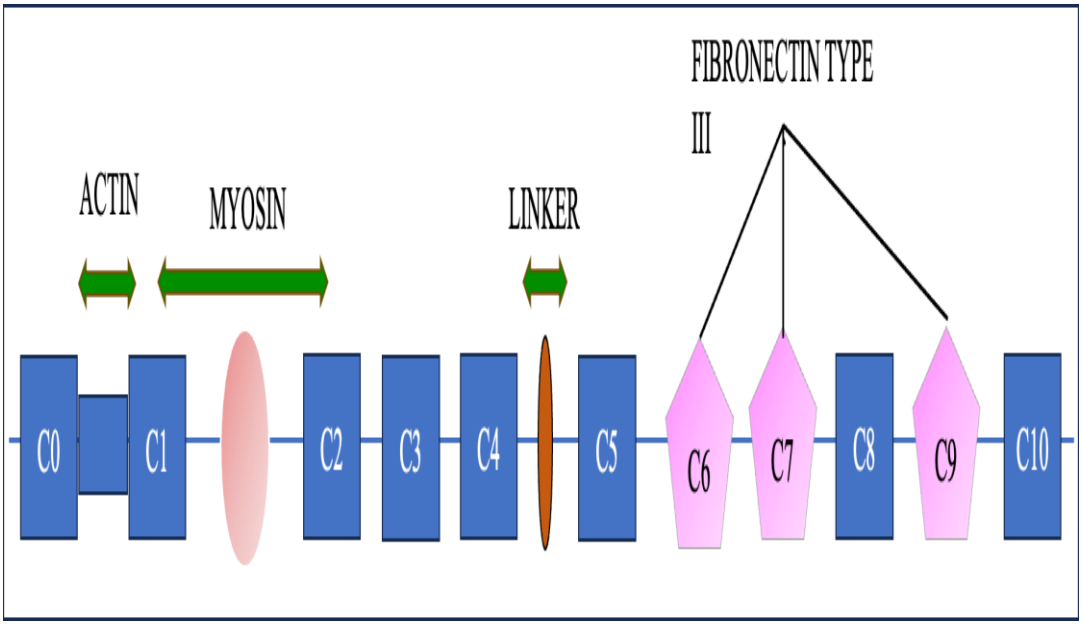


Figure 2.12: Representation of the cMyBP-C protein structure includes eight immunoglobulin-like domains (C0, C1, C2, C3, C4, C5, C8, C10) and three fibronectin type III domains (C6, C7, C9). A distinctive orange oval symbolizes the linker region between the C4 and C5 domains. (Tudurachi et al., 2023).

Function of *MYBPC3*

Heart (cardiac) muscle cells contain cardiac myosin binding protein C (cMyBP-C), which is produced by the MYBPC3 gene. This is associated with thick filaments and functions as a structural and regulatory component of cardiomyocyte contraction (Flavigny et al., 2003). The sarcomere, the basic component that makes up muscular contraction, is linked to cMyBP-C in these cells. The filaments that make up sarcomeres are both thick and thin. Muscle contraction requires the adhesion and subsequent release of overlapping thick and thin filaments (Carrier et al., 1997). The heart's muscle propels blood to the rest of the body regularly. cMyBP-C is binded to thick filaments in heart muscle sarcomeres to prevent early degradation. Phosphate groups are molecules connected to cMyBP- C, and when they are withdrawn, cMyBP-C and the thick filament proteins break down next (Bhola et al., 2018). Research indicates that cMyBP-C may have two roles. Its first role is to act like a molecular ruler. It regulates the distance between thick and thin filaments. The other role is as a regulator of actomyosin interaction through its affiliation with F-actin and the myosin II neck region (S2) (Dhandapany et al., 2009). While the C-terminal portion of cMyBP-C is believed to have a structural anchoring role, the N-terminal portion of the protein acts as a crucial

regulator of contractile function. The C-terminal portion binds to the thick filament and is necessary for integration into the sarcomere in domains C7–C10. The light meromyosin (LMM) region of myosin rods and the C10 domain of cMyBP-C interact to generate the thick filament's support structure. It has been demonstrated that the domains C8–C10 attach to titin immunoglobulin domains that are repeated in the thick filament's C zone every 42 nm (Kuster and Sadayappan, 2014).

MYBPC3 Polymorphism

MYBPC3 polymorphism has a 25-base pair deletion (MYBPC3 Δ 25bp) in intron 32 (Dhandapany et al., 2009). The C-terminal region of cMyBP-C has 58 new amino acids instead of 65 wild-type amino acids in C10 domain due to *MYBPC3 Δ 25bp* (Kuster and Sadayappan, 2014). Missense amino acids are incorporated at the protein's C-terminal region due to the loss in intron 32, which skips the downstream exon 33. Exon 33 is skipped due to the 25 bp intronic deletion,and missense amino acids are incorporated at the C-terminal (Waldmüller et al., 2003). The final 65 amino acids of the C10 domain of C-terminal of cMyBP-C are replaced with a novel sequence of 58 residues (cMyBP-Cc10mut), which moves the stop codon to the 3' UTR by this mutation, skipping exon 33 and resulting in a frame shift. After passing through exon 34 across a portion of the 3′-UTR, translation stops (Sadayappan et al., 2020). The mutant protein has been demonstrated to be incorporated into myofibrils, which may lead to sarcomere collapse (Flavigny et al., 2003). The influence of secondary risk factors and the late development of symptoms could pose a long-term threat to carriers. However, gene-based insights into pathophysiology may enable the identification of other phenotypes linked to mutation and more subtle clinical presentations. It has also been shown that mortality and LV ejection fraction (LVEF) are closely related (Srivastava et al., 2011). Below is the diagram depicting 25bps deletion; as,

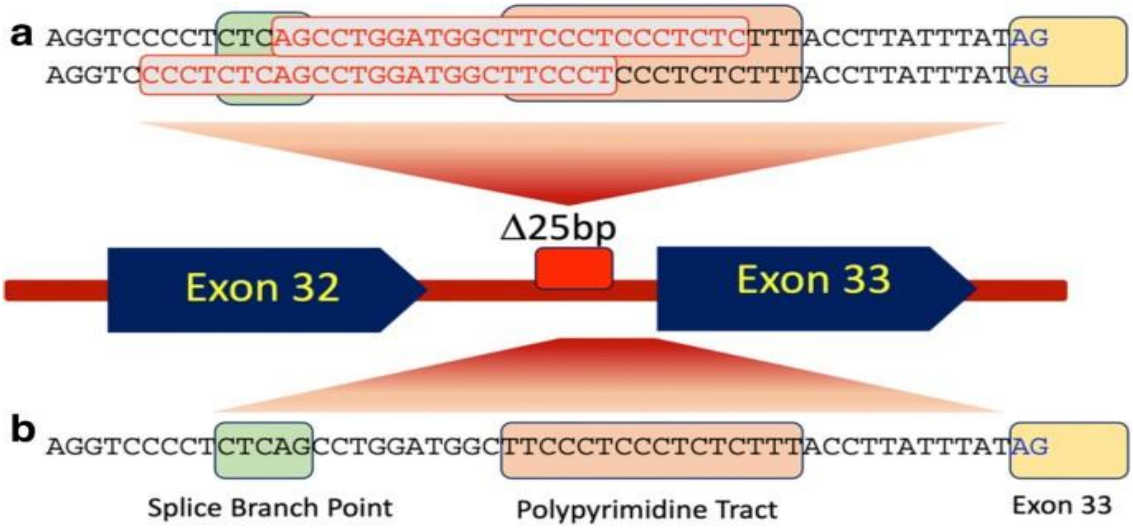


Figure 2.13: Genotype of *MYBPC3Δ25bp* in intron 32 of the *Myosin binding protein-C* gene. *(a) Two different deletions of 25 bps are displayed, and both have similar results. (b) The position of the polypyrimidine track and splice branch point at the intersection of intron-32 and exon-33 splicing* (Arif et al., 2020).

Mutant *MYBPC3* is translated into mRNA in human patients with truncation mutations; nevertheless, altered cMyBP-C proteins are undetectable, and overall, cMyBP-C in the sarcomere is considerably decreased. The interaction change with *LMM* is the most expected result of the cMyBP-CC10 mutation (Kuster and Sadayappan, 2014). Theoretically, since cMyBP-Cc10mut does not bind to myosin LMM, it will be eliminated by the ubiquitin proteasome system (Srivastava et al., 2011).

In patients with hypertrophic cardiomyopathy (HCM) who had single pathogenic variations that were either *MYBPC3* (n=48, 76%) or *MYH7* (n=15, 24%), the genotype-phenotype connection was examined in this study. The symptom with the greatest frequency in the *MYBPC3* group was dyspnea (44%), whereas palpitations were more common in the *MYH7* group (33%). In the *MYBPC3* group, a favourable family history was more common, but in the MYH7 carriers, atrial fibrillation was more common. Higher-risk HCM phenotypes were supported by the fact that *MYH7* mutations were often linked to higher clinical severity (Velicki et al., 2020).

2.6.4 BCL2 Associated Athanogene 3/ BAG Co-chaperone 3 (BAG3) Gene

Cytogenetic location of *BAG3*: The BAG3 gene is found on the long arm of chromosome 10 at position 10q26.11.

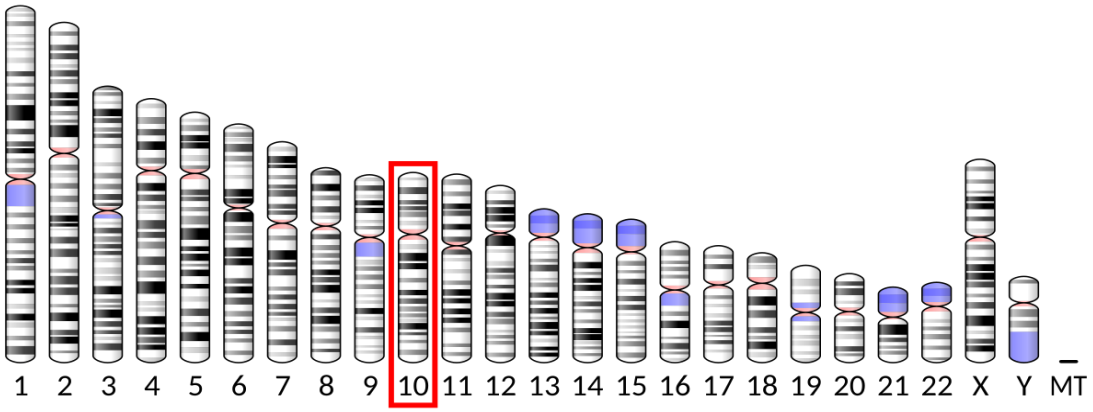


Figure 2.14: Ideogram view of BAG3 gene (From Ensemble database)

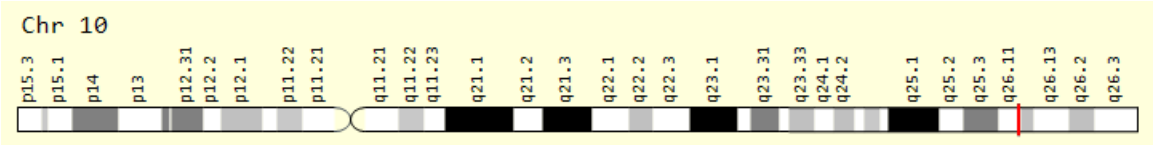


Figure 2.15: Cytogenetic location of BAG3 gene [From Gene cards].

BAG3 is a protein present across all mammalian tissues, but it is particularly abundant in the heart and skeletal muscle, where it plays essential roles in cellular function and survival (McCollum et al., 2010). The level of *BAG3* expression is sensitive to various stress factors that can impact cells, including ischemia (a lack of blood flow), viral infections, heat shock (extreme temperature increases), oxidative stress (damage caused by reactive oxygen species), serum starvation (lack of nutrients), and exposure to elevated temperatures (Pagliuca et al., 2003; Wang et al., 2008).

Research conducted in recent years, particularly by Homma et al. (2006), has established that *BAG3* is critical for maintaining the health and stability of heart muscle cells (myocytes). This is referred to as myocardial homeostasis, which is vital for proper heart function. In neonatal myocytes (young heart cells), *BAG3* is primarily found in the cytoplasm, which resides within the cell's interior. However, as these myocytes mature into adult myocytes, the localisation of *BAG3* shifts. In adult myocytes, *BAG3* is located at the sarcolemma (the cell membrane of muscle cells) and along the transverse tubules (extensions of the cell membrane that help to facilitate heart muscle

contraction). Sarcomeres are the essential building blocks of muscle contraction in both heart and skeletal muscle cells. *BAG3* plays a crucial role in offering structural support, ensuring the integrity of the sarcomere, which is essential for proper muscle contraction. *BAG3* plays a significant role in regulating the heart's response to adrenergic stimulation, including hormones such as adrenaline, and is crucial for the excitation–contraction coupling. The β1-adrenergic receptor is essential for transmitting signals that increase heart rate and contractility, while L-type Ca^{2+} channels regulate calcium entry into the cells, which is crucial for muscle contraction. This process connects the electrical stimulation of heart cells to their physical contraction. *BAG3* interacts with the β1-adrenergic receptor and L-type calcium channels to facilitate effective calcium handling and maintain contractility (Feldman et al., 2016). Thus, *BAG3* contributes to the proper functioning of the heart, especially under stressful conditions, by helping to ensure that the signalling pathways result in effective muscle contractions. Mutations in this gene have been linked to serious diseases, including myofibrillar myopathy and dilated cardiomyopathy, which can severely affect muscle and heart function.

Structure of *BAG3*

Bcl2-associated athanogene 3 (*BAG3*) is a cochaperone protein having 575-amino acid essential for cardiac muscle function, specifically in preserving sarcomere integrity and protein homeostasis (Knezevic et al., 2015). In terms of structure, *BAG3* is made up of a WW domain at the N-terminus, a proline-rich (PXXP) motif at the C-terminus, and a *BAG* domain at the C-terminus (Qu et al., 2022). These domains serve a crucial function in protein quality regulation through chaperone-assisted selective autophagy (CASA), facilitating interactions with molecular chaperones such as Hsp70 and small heat shock proteins (sHSPs) (Butt et al., 2022) These connections are broken by mutations in *BAG3*, such as the P209L variation, which results in dilated cardiomyopathy (DCM) symptoms such protein aggregation, sarcomere disarray, and decreased heart contractility (Martin et al., 2023). *BAG3* mutations affect sarcomeric protein turnover, which leads to contractile failure and an increased vulnerability to apoptosis, based on findings using genome-edited induced pluripotent cardiomyocytes (McDermott-Roe et al., 2019). These results highlight how important *BAG3's* structural domains are for heart function and how they cause DCM (Liu et al., 2021).

BAG3 Polymorphism

A study published that identifies a notable genetic alteration characterised by a large deletion of 8733bp in exon 4 of the BAG3 gene. The 428 single-nucleotide variations found by the WES resulted in splice site, missense, or nonsense changes. These mutations were observed to be inherited in an autosomal dominant manner within a family suffering from dilated cardiomyopathy, a condition that results in the enlargement and weakening of the heart, compromising its blood-pumping capability (Norton et al.,2011). Another study by Selcen (2011) reported symptoms including skeletal muscle weakness and neurological issues in children with similar genetic changes, while the family studied by Norton did not show these additional symptoms.

A unique causative variant in the BAG3 gene through a genome-wide association study involving DNA from 1,199 individuals with dilated cardiomyopathy and a comparable number of control subjects. Subsequent sequencing of DNA from a substantial group of probands revealed additional *BAG3* variants. A mutation in the BAG3 gene might not affect the heart's function during normal daily life. However, exposure to stressful events could make one more prone to left ventricular problems (Villard et al.,2011).

Recent studies by Lee et al. (2012) have identified alterations in Bcl-2-associated athanogene-3 (BAG3). This anti-apoptotic protein is a co-chaperone of heat shock proteins (HSPS), which is associated with familial dilated cardiomyopathy (FDC). These findings highlight the critical role of BAG3 in maintaining cardiomyocyte function and suggest its involvement in the pathogenesis of inherited forms of DCM.

The BAG3 gene has been identified as a significant cause of dilated cardiomyopathy (DCM). A study of 90 Polish DCM patients found five BAG3 mutations, including one known (p.Glu455Lys) and four novel mutations, along with a large deletion affecting exons 3–4. Among six probands, 21 affected relatives carried BAG3 mutations. Clinical data suggested acute DCM onset in mutation carriers, often triggered by infections. These findings highlight the importance of BAG3 mutations in DCM pathogenesis and genetic counselling (Franaszczyk et al., 2014).

A significant reduction in BAG3 protein levels in the myo-cardial tissue of patients with end-stage dilated cardiomyopathy (DCM) who required heart transplantation, regardless of the absence of a familial history of myocardial disease. These findings suggest that alterations in BAG3 expression may contribute to the pathogenesis of non-familial forms of DCM. Moreover, the study indicates that reduced BAG3 protein levels are observed not only in individuals harbouring BAG3 mutations but also in patients with end-stage heart failure, highlighting the broader significance of BAG3 deficiency in myocardial dysfunction (Feldman et al., 2014).

The reducing BAG3 levels by 55% weakened heart muscle cell contractions and lowered calcium levels when stimulated with isoproterenol (a drug that increases heart activity). The study also found that BAG3 interacts with important proteins in heart cells, including sodium-potassium pumps and calcium channels, which help regulate heart contractions. These outcomes highlight that BAG3 plays a key role in controlling heart muscle function, especially in response to signals that increase heart activity (Knezevic et al., 2016).

The inspection of a large Spanish family with dilated cardiomyopathy (DCM) to find new genetic variants linked to the condition. About half of all cases of chronic heart failure were hereditary, and DCM was one of the leading causes of this condition. Of 100 family members, 32 had a frameshift mutation in the BAG3 gene (p.H243Tfr64). The fact that 10 of them were asymptomatic and 21 displayed clinical symptoms of DCM illustrates the variation in the disease's presentation. The findings imply that, especially in younger individuals, in BAG3 is a recently discovered pathogenic variation associated with a more severe type of DCM. This study emphasises how crucial genetic screening is for identifying at-risk individuals early and implementing preventative measures (Toro et al., 2016).

BAG3 mutations are commonly linked to dilated cardiomyopathy (DCM), making it an important gene for genetic screening in affected families. BAG3 also plays a key role in autophagy regulation, especially during oxygen level changes. Researchers prefer green, fluorescent and red fluorescent protein (GFP-RFP) reporter genes to measure autophagy activity, helping to understand its function and potential as a treatment target (Esslinger et al., 2017).

The genetic characteristics are associated with common types of heart failure (HF) and are sought to validate prior genetic associations. Using a case-control design, researchers examined 14 potential genes in 799 HF patients and 1,529 healthy controls. The study discovered that the C allele of BAG3 gene was substantially related with a decreased incidence of idiopathic dilated cardiomyopathy ($p = 0.0005$), which confirmed previous findings. No other significant relationships were found, including in genome-wide analysis. These findings support the BAG3 variant's protective effect in idiopathic dilated cardiomyopathy (Denus et al., 2020).

2.7 Telomere Length with Cardiomyopathy

Telomeres consist of repeating nucleotide stretches found at ends of eukaryotic chromosomes that act as protective caps to maintain genomic integrity during cell division. The end-replication issue causes telomeres to shrink each time a cell divides. This shortening is an involuntary feature of the cell cycle that also estimates a cell's number of divisions and hence biological age (Blackburn et al., 2006). The reduction in the length of telomeres as a cell undergoes the successive stages of its lifecycle is essential for DNA replication in eukaryotes (Victorelli and Passos, 2017). So, we called this gradual shortening a defining feature of ageing, and it has significant implications for several illnesses, including heart conditions. Over time, telomeres shorten to an extent where the cell can no longer divide properly. This leads to either apoptosis (cell death) or senescence, the cell's cessation of growth. This shortening of telomeres is a natural part of ageing and is also linked to diseases like heart conditions and other age-related disorders.

Telomere deterioration has been linked to the cause of cardiovascular disorders. Telomere shortening in vascular endothelial cells and cardiomyocytes may affect cellular function and survival, leading to hypertension, cardiovascular disease, and heart failure (Serrano and Andres, 2004). Furthermore, telomere disruption causes the development of telomeres to significantly lose their capacity to adequately protect chromosomal ends when they are critically short and incapable of holding the ends of chromosomes, which triggers DNA-damaging reactions (Zhan and Hagg, 2019). The two primary biological outcomes of this damage response are apoptosis or programmed cell death and senescence, where the cell continues to be metabolically active but stops dividing (Akincilar et al., 2016). Due to the negative impact on the heart's structure and

decreased ability to regenerate cardiac tissue, both results significantly increase the risk of cardiac dysfunction. Thus, it is becoming more widely recognised that telomere shortening plays a significant role in developing cardiovascular disorders.

It has been suggested that leukocyte telomere length (LTL) serves as a biomarker for cardiovascular risk and systemic biological ageing. Salih et al. (2024) showed an association between genetically determined shorter LTL and severe cardiac anatomical alterations, such as lower ventricular volumes. They decreased left ventricular mass in a Mendelian randomisation study. According to these results, LTL may be used as a simple biomarker to identify those at increased risk of cardiomyopathy and other heart dysfunctions.

The prospective research on African American and white American individuals was conducted and found that CHD patients had an average telomere length (TL) of 4.80 kb, compared to 4.90 kb for controls. Participants' average was 74 years, with 49% being men. (Njajou et al., 2009).

The interaction between coronary heart disease (CHD) and leukocyte telomere length (LTL) in the United States. A lower telomere length (LTL) may be linked to a higher risk of CHD, since the study indicated that males who developed CHD had shorter average telomere lengths (3.41 kb) than healthy controls (3.52 kb). This strengthens LTL's potential as a cardiovascular risk biomarker in this cohort (Zee, 2010)

The Telomere length inheritance in 962 people between the ages of 0 and 102. Strong father-child correlations ($r = 0.454$, $p < 0.001$) indicate a dominating male contribution, with lesser maternal connections. There was also a moderate association between grandparents and grandchildren ($p = 0.013$). Paternal impact was much stronger ($p = 0.012$), suggesting a maternal effect in daughters. Correlations decreased with age ($p = 0.022$), showing that environmental factors change with time (Nordfjäll et al., 2010).

The link between length of telomere and ischemic heart disease (IHD) among 105,055 Copenhagen residents, including 17,235 IHD patients. Telomere length was measured in 66,618 people, and genetic variations in OBFC1, TERT, and TERC were investigated. A 200-base pair shorter telomere was associated with a 1.02 hazard ratio for IHD. Genetic data from up to 184,967 people (including CARDIOGRAM) revealed

that these variations were related to shorter telomeres and higher IHD risk, with a 200-base pair genetically dictated decrease corresponding to an odds ratio of 1.10, confirming a causative relationship (Scheller et al., 2016).

The severity and course of the disease are correlated with telomere shortening, indicating a potential role in the pathophysiology of cardiomyopathy. Moreover, telomere disruption can trigger DNA damage reactions, resulting in cardiomyocyte fibrosis and death, two characteristics of cardiomyopathic remodelling (Booth and Charchar, 2017). When the role of telomere biology in cardiovascular health becomes clear, treatment options become possible. Telomere shortening and its harmful impact on the cardiovascular system may be lessened by using antioxidant therapy, lifestyle changes (such as consistent exercise and a balanced diet), and telomerase activation (Booth and Charchar, 2017). Shorter telomeres have been linked to several types of cardiomyopathies in several studies. In contrast to healthy people, patients with hereditary cardiomyopathies, such as dilated and hypertrophic cardiomyopathy, have substantially shorter telomeres in their cardiac tissues (Chang and Blau, 2018).

Analysis of 8,892 participants showed that longer telomere length was linked to higher HDL and lower fat mass, HbA1c, and CRP in men. Women showed a negative trend only for HbA1c. Men with the longest telomeres had a 38% lower risk of metabolic syndrome, highlighting a stronger protective effect in males. (Mazidi et al., 2018).

The literature review emphasises that cardiomyopathy is a complex condition with several risk factors and the effect of different genes and their polymorphism. This review serves as an inspiration for additional research focused on enhancing cardiac health outcomes. Despite the advancements in understanding the genetic and non-genetic determinants of cardiomyopathy, several gaps remain, particularly in the context of the Jammu region. First, there is a lack of population-specific genetic studies to identify unique variants and their clinical implications. Second, the impact of environmental factors, such as high-altitude living and air pollution, on cardiomyopathy in this region remains poorly understood. Third, there is a need for longitudinal studies to evaluate the interplay between genetic and non-genetic factors in the development and progression of cardiomyopathy. To fill in the gaps and provide more potent preventative and treatment plans. Future research should address these gaps through

comprehensive genetic screening, environmental assessments, and lifestyle interventions.

CHAPTER-3
HYPOTHESIS

3.1 Hypothesis of the Study

The Jammu and Kashmir has a complex cultural and genetic environment, with three separate ethnic groups: Kashmiri (Kashmir Valley), Dogra (Jammu), and Ladakhi. Complex gene–environment interactions impact the onset and appearance of cardiovascular illnesses. A person's genetic predisposition, ethnic origin, and lifestyle variables all influence their susceptibility to Cardiomyopathy (CM). Numerous studies conducted worldwide have shown that there is considerable genetic variability linked to cardiomyopathy. Certain genetic variations have been linked to cardiovascular risk in several Indian states. However, regarding genetic research on cardiomyopathy, the Jammu region of Jammu and Kashmir is still unexplored. The present study aims to find out if certain candidate gene polymorphisms are linked to CMs in people from the Jammu area. Polymorphisms in the *ACE* gene, the *MYH7* gene, the *MYBPC3* and *BAG3* are specifically the focus of the investigation. The pathogenesis of disease has been linked to these genetic markers, which are thought to raise the risk of disease (Gupta et al., 2013). Finding these polymorphisms might help identify high-risk patients early, allowing for individualised treatment plans and lowering the socioeconomic cost of treatment.

An increased risk of cardiovascular disease has been associated with polymorphisms in the ACE gene, namely the insertion/deletion (I/D) variations. Higher ACE levels are linked to the D allele, and this can lead to increased vascular resistance and hypertension. These alterations cause oxidative stress, interfere with the renin-angiotensin-aldosterone system (RAAS), and initiate several pathological alterations that negatively impact the structure and function of the heart. *MYH7* and MYBPC3 encode sarcomeric proteins, which are essential for the heart muscle's ability to contract. *MYBPC3* and *MYH7*, respectively, encode the cardiac myosin-binding protein C and β-myosin heavy chain, which are necessary for the heart to contract and relax in synchronisation. Co-chaperone protein *BAG3* is essential for preserving sarcomere stability, protein quality regulation, and cardiac cytoskeletal integrity, especially under stressful situations. Any changes in these genes may disrupt contractile mechanism and affect sarcomere integrity, which often results in dilated or hypertrophic cardiomyopathy, sarcomere disorder and cardiomyocyte degeneration.

The substantial data gap concerning non-genetic (modifiable) risk variables, including food, physical activity, tobacco use, and comorbidities, about the prevalence of cardiomyopathy in Jammu and Kashmir is also addressed in this study, in addition to genetic determinants. A secondary goal of the research is to evaluate the correlation between these non-genetic risk variables and specific Cardiomyopathy phenotypes common in the Jammu area. To develop comprehensive public health policies for the prevention, early detection, and successful management of cardiovascular diseases in this population, it is necessary to understand the roles played by both hereditary and environmental factors.

CHAPTER-4
AIM AND OBJECTIVES

4.1 Aim of the Study

Cardiomyopathy alters the structure of the heart muscle, causing it to be harder for the heart to pump blood efficiently and raising the risk of heart failure and sudden cardiac death. The outcomes of numerous studies that have examined the genetic and non-genetic factors that contribute to cardiomyopathy have been mixed. Interestingly, no studies have examined the genetics of patients with Indian cardiomyopathy, and none have examined the populations of Jammu and Kashmir (J&K). In particular, the Jammu population has not been thoroughly studied to determine the pertinent genetic and non-genetic components. To fill these gaps, this study will genotype and sequence the genes of cardiomyopathy patients and healthy controls in the J&K region to find mutations associated with the condition**.** The aim of current investigation is to evaluate the impact of polymorphisms in cardiomyopathy genes on this population and investigate the effects of these mutations on important gene networks at the molecular level, which may contribute to the illness. The effects of environmental and lifestyle factors, such as high altitude, dietary practices, and socioeconomic level, on the course and clinical results of the disease have also not been thoroughly studied. The necessity for integrated multi-omics research to better understand the molecular networks involved is highlighted by the lack of knowledge regarding the relationship between genetic variants and treatment response. By identifying genetic variants within these populations, customised therapeutic methods for high-risk individuals may be developed, improving quality of life and lowering the disease's social and economic burden

4.2 Objectives

The current research will be conducted with the following goals in mind:

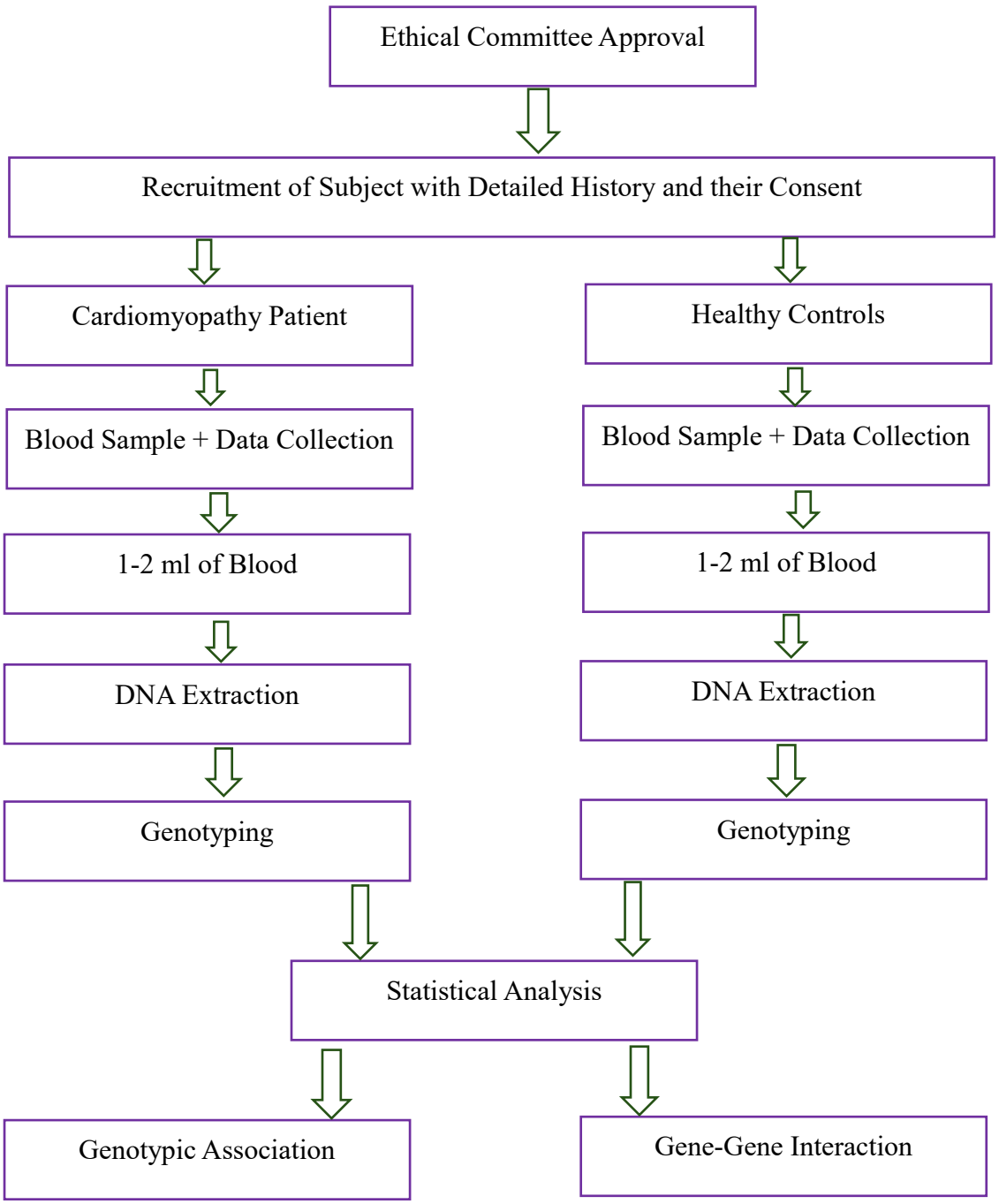
1. To identify clinically positive confirmed cardiomyopathy patients and enrol them.
2. To find out the genotypic and allelic frequency of the selected candidate genes of cardiomyopathic patients.
3. To find out the association of selected candidate gene polymorphism with the susceptibility of cardiomyopathy.
4. To evaluate the association of non-genetic risk factors with the susceptibility of cardiomyopathy.

CHAPTER-5
MATERIAL AND METHODS

5.1 Research Plan

The increasing prevalence of cardiomyopathy has encouraged researchers to investigate its genetic basis. In the present study, we observed both genetic and non-genetic risk factors in relation to cardiomyopathy patients. Therefore, this case-control study was conducted to examine the association between SNPs in various genes with cardiomyopathy in the Jammu population of the UT Jammu and Kashmir (J&K), India.

Research Design



5.2 Ethical Authorization

The current study design was duly approved by the Government Medical College Jammu Institutional Ethics Committee with reference number IEC/GMC/2022/1143, ensuring compliance with established ethical protocols. Each participant was informed of the nature and scope of the current study. The data and blood sample were collected after prior informed written consent from each study participant/attendant, or guardian (for incompetent participants).

5.3 Study Population and Area

The current study examined 250 confirmed Cardiomyopathy cases and 500 unrelated healthy controls from various areas of the Jammu region in Jammu and Kashmir (J&K), UT. The Cardiomyopathy cases were enrolled from the Outpatient Department of Cardiology at Superspeciality Hospital (SSH), Government Medical College, Jammu. The controls were enrolled from the Blood Bank, Shri Maharaja Gulab Singh (SMGS) Hospital, individuals attending the hospitals SSH and University of Jammu. The inclusion and exclusion criteria for selected cases and controls are included in Tables 5.1 and 5.2, respectively. Further, the study was conducted at the Institute of Human Genetics (IHG), University of Jammu.

Table 5.1: Inclusion and Exclusion Criteria for Cardiomyopathy Patients.

Inclusion Criteria	Exclusion Criteria
1. Clinically diagnosed cardiomyopathy, arrhythmia, DCM, HCM & heart block. 2. Both male and female subjects. 3. ECG and Echocardiography will be used to evaluate suspected individuals. 4. Left ventricular enlargement (LVE) ≥13 mm, or >2 standard deviations corrected for body surface area. 5. An LVEF of 40% indicates a reduced ejection fraction (Rai and Ahmad et. al., 2009).	1. Individuals with autoimmune diseases are excluded to avoid confounding effects on myocardial morphology. 2. Patients diagnosed with any form of malignancy are excluded to eliminate potential influences treatment-induced cardiac changes. 3. Exclusion of individuals with coronary artery disease (CAD) to avoid confounding effects of ischemia-related changes on myocardial wall thickness. 4. Patients with advanced stages of chronic renal failure are excluded due to the associated risk of uremic cardiomyopathy or other cardiac complications. (Rai and Ahmad et. al., 2009).

Table 5.2: Inclusion and Exclusion Criteria for Controls.

Inclusion Criteria	Exclusion Criteria
1. Participants with no medical history of major illnesses and without any diagnosed heart conditions. 2. Age-matched individuals 3. Participants with blood pressure levels within the normal physiological range. 4. Both male and female subjects. 5. Individuals with blood glucose levels within the normal range.	1. Participants with blood glucose levels indicate hyperglycemia or hypoglycemia. 2. Patients undergo treatment for any condition that may influence their cardiovascular health. 3. Individuals with abnormal lipid profiles, including elevated LDL, low HDL, or elevated triglyceride levels. (Bharti et al., 2025)

5.4 Data Collection from Subjects

A well-structured questionnaire was administered to gather comprehensive information from each participant according to CCD guidelines with slight modifications. The questionnaire covered key parameters such as gender, age, height, marital status, weight, residence place, religion, history of cardiomyopathy, smoking/tobacco and alcohol consumption habits, dietary patterns, physical inactivity, and family history of genetic diseases (CDC, Government Agency, Rahman et al., 2013). Additionally, anthropometric measurements, including Body Mass Index (BMI), were recorded. A copy of the questionnaire and the consent form are provided in the Appendices.

Anthropometric and Physiological measurements: Body Mass Index (BMI) was calculated using the formula weight, i.e., (kg) / height² (m^2) and categorised according to World Health Organization (WHO) recommendations for Asians (WHO Expert Consultation, 2004). The Pulse rate (PR) of an individual was calculated by touching the radial artery at the wrist and counting the number of pulses over a one-minute period. To calculate pulse pressure (PP), use the formula PP = Systolic Blood Pressure (SBP) – Diastolic Blood Pressure (DBP) (Chobanian et al., 2003). Psychological factors such as stress, tension, headaches, and anger were assessed through direct interviews. Stress was classified into different categories based on its source, including financial stress, family-related stress, work-related stress, educational stress, depressive mood, and health-related stress. For patients unable to provide information due to illness or

other limitations, relevant details were obtained through interviews with their guardians.

5.5 Blood Sample Collection

Blood samples of patients shall be collected from the district hospitals of the Jammu region of UT, Jammu and Kashmir. Two to three mL of blood shall be collected from each patient with informed/written consent of the individual at the time of enrollment. Also, for comparative analysis, blood samples will be aspirated from healthy controls. The collected blood shall be stored in EDTA-coated tubes at -20°C until DNA isolation.

5.6 Extraction of Genomic DNA by Using Phenol Chloroform Method

Protocol: DNA extraction from whole blood was carried out by using Phenol: Chloroform method as given by Sambrook and Russel, (2001) with slight modifications. Blood samples obtained from the study participants, stored at -20°C, were thawed and kept at room temperature before the extraction of DNA. Lymphocytes from whole blood were extracted by using a hypotonic solution and then added ELB (erythrocyte lysis buffer) (155mm NH4Cl, 10mm KHCO3, 0.1mm EDTA (Himedia) with minimal lysing effect on Lymphocytes in a microcentrifuge tube (Eppendorf). Three volumes of erythrocyte lysis buffer (ELB) (1 M NaCl, 10 mm Tris, 2 mm EDTA Himedia) were added to the blood sample, followed by vortex for 5 minutes and then the mixture was centrifuged (Fisher Scientific) at 7000 rpm (4°C) for 15 minutes. After its supernatant was discarded. To pellet, three volumes of RBC lysis buffer (ELB) were added to the pellet of sample, followed by vortex and inverted for 5 minutes, and then centrifuged at 7000 rpm (4°C) for 15 minutes. Repeat the process two or three times till clear pellets and supernatant were obtained. After the final wash, the supernatant was discarded, and to the pellet, add 500ul of PBS [Na_2HPO_4, KH_2PO_4, KCL, NaCl (Himedia)], 400ul cell lysis buffer (lysis buffer-II), 20% SDS and 2-3ul of proteinase K (Himedia). Vortex the sample to dissolve the pellet, then incubate overnight at 37 °C. An equal volume of Tris equilibrated phenol (Himedia) is added. Centrifuge the sample at 7000 rpm (4°C) for 15 minutes. The supernatant was transferred to another microcentrifuge tube. Transfer the supernatant to a 2 ml microcentrifuge tube and subsequently add Chloroform and Isoamyl-alcohol (24:1). The mixture is centrifuged for 10 minutes at 7000 rpm (4°C). Two layers of mixture appear, upper aqueous and

lower organic. Aspiration was used to extract the upper layer into a 1.5 ml microcentrifuge tube. To the isolated supernatant (upper aqueous layer), add twice the amount of absolute ethanol (Analytical Reagents), invert gently for some time. Incubate the mixture for 30 minutes at -20°C. Centrifuge it for 15 minutes at 7000 rpm (4°C). Supernatant discarded and 70% ethanol added to pellet with gentle tapping. Centrifuge it for 15 min at 7000 rpm (4°C). Repeat this step at least three times for proper washing. The pellet was placed in a laminar air flow chamber overnight for air dried. Resuspended the dried pellet in 50ul nuclease-free water or 1X TE [10mM EDTA, 100mM- Tris-Cl (Himedia)] buffer. Store the DNA at -20°C or -80°C for further processing.

5.7 Qualitative and Quantitative Analysis of Isolated Genomic DNA

5.7.1 Agarose Gel Electrophoresis

The quality of the isolated genomic DNA was examined by agarose gel electrophoresis using a 0.8 % agarose gel. 100 ml of 1x TAE was measured in a graduated cylinder, and 0.8g of electrophoresis-grade agarose (Sigma-Aldrich) was weighed. Both were mixed in a 250 ml flask, and the agarose was melted slowly without boiling by heating in a microwave. It swirled slowly till it became a homogeneous solution. The molten agarose was cooled to 50-55°C, and then 0.8µl of Ethidium-Bromide (0.5µg/ml, Merck) was added and mixed thoroughly. The gel casting tray and comb were cleaned with 70% alcohol, and the comb was then put into the slot of the gel casting tray. The molten agarose was poured slowly and allowed to solidify. Solidified gel was then placed in the gel tank, filled with 1x TAE/1x TBE buffer. Each DNA sample (3µl) was mixed with 1µl of 1x loading dye (bromo-phenol blue, EDTA, glycerol) and before being loaded on to the gel. Electrophoresis was performed at 100 volts for one hour. The bands of DNA were visualised under a UV transilluminator (Bossù,1999). The amount and quality of an aliquot of DNA were then assessed by comparing its intensity with DNA standards of known characteristics (Figure 14).

5.7.2 Spectrophotometry

Spectrophotometry was used to measure the amount of extracted DNA. The DNA concentration was determined by employing the following formula: Concentration (µg/ml) = 50 × OD_{260} × dilution factor. This calculation was based on the

idea that an absorbance of 1.0 at 260 nm (OD_{260}) corresponded to 50 µg/ml of double-stranded DNA. By computing the absorbance ratio at 260 and 280 nm (OD_{260}/OD_{280}), the purity of the DNA was checked. Pure DNA was indicated by a ratio of around 1.8. Protein pollutants were present when the ratio was less than 1.8, whereas RNA contamination was suggested by ratios greater than 1.8. A DNA workable solution was created after qualitative and quantitative evaluations.

5.8 Genotyping of Selected Candidate Genes

The genotyping of the selected candidate gene was conducted by using polymerase chain reaction, followed by restriction fragment length polymorphism (PCR-RFLP) technique. PCR–RFLP method involves detection of polymorphism based on site-specific restriction enzyme digestion of PCR amplified DNA template, as in the sample, followed by gel electrophoresis. If a genetic variation is present, a unique digestion pattern can be observed, suggesting gain or loss of restriction sites in specific alleles. Four candidate gene polymorphisms were selected for the study and are described in Table 5.3.

Table 5.3: Candidate Gene Polymorphisms Selected for the Present Study.

S. No	Gene Polymorphism (Rs ID)	Chromosome location	Amino acid change	Genotyping technique
1.	*ACE* (rs1799752)	Chromosome 17	Insertion/ deletion	PCR
2.	*MYH7* (rs397516208)	Chromosome 14	G>A Missense variant	PCR-RFLP
3.	*MYBPC3* (rs36212066)	Chromosome 11	25bp deletion	PCR
4.	*BAG3* (rs2234962)	Chromosome 10	T/C Missense variant	TaqMan Assay

Table 5.4: Candidate Gene Polymorphisms and their Primer Sequences.

Gene polymorphisms	Primers	Primer Sequence	Amplicon (base pairs)
ACE (I/D) **(Rani et al., 2017)**	Forward Reverse	5'- CTGGAGACCACTCCCATCCTTTCT - 3' 5'- GATGTGGCCATCACATTCGTCAGAT- 3'	**190**
MYH7-Taq 1 **(Villard et al., 2005)**	Forward Reverse	5'- ATCCTCCCCACCCTCTGC - 3' 5'- GAGGATGGCTCTGGCCTCT - 3'	**299**
MYBPC3 **(Simonson, et al., 2010)**	Forward Reverse	5'-GTTTCCAGCCTTGGGCATAGTC -3' 5'-GAGGACAACGGAGCAAAGCCC -3'	**403**
BAG3 **(Villard et., 2011 and GWAS)**	TaqMan probe		

5.8.1 Polymerase Chain Reaction (PCR)

PCR is a molecular technique which is used to amplify a single or a few copies of a DNA and generate thousands to millions of copies of that DNA segment. The technique relies on thermal cycling, comprising cycles of repeated heating and cooling the reaction to melt DNA and enable enzymatic replication. Primers, which are short, single-stranded DNA oligonucleotides that complement certain sequences within the target area, as well as a thermostable DNA polymerase, are required for the selective and exponential amplification of the targeted DNA fragment during PCR. For the present research work, primer sequences for the selected candidate genes were retrieved from the literature (Table 5), and the amplification efficiency of primers was confirmed by in silico PCR (UCSC genome browser, http://www.genome.ucsc.edu). Genomic DNA was amplified by the polymerase chain technique with the help of 96-96-well thermal cycler machine (Proflex, Applied Biosystems by Life Technology, Singapore), using allele-specific oligonucleotides procured from Eurofins. The details of the mastermix used for amplifying the desired fragments for genotyping of selected polymorphisms are represented in Table 3.5. The PCR amplification profile pattern for selected polymorphisms is given in Table 3.4. The PCR amplification products were

electrophoresed on a 1.5% (w/v) agarose gel in 1× TAE buffer. After electrophoresis, DNA bands were seen under ultraviolet (UV) light after being stained with a nucleic acid dye (ethidium bromide or SYBR Safe). The concentration of the amplified PCR products was determined by comparing the fluorescence intensity of the sample bands to that of known fragment concentrations in a 100 bp or 50 bp DNA ladder, which served as a reference DNA.

Table 5.5: Composition of PCR Master-Mix for Amplifying Candidate Gene Polymorphism.

Composition of 1X Reaction	Working Concentration	*ACE* (µl)	*MYH7* (µl)	*MYBPC3* (µl)	*BAG3* (µl)
DDW	-	7.5	9	5	1
2X PCR Master mix	1X	10	12	12	1.25
F.P. (100 pmol/µl)	0.1-1µM	0.5	0.5	0.5	Probe = 0.25
R.P. (100 pmol/µl)	0.1-1µM	0.5	0.5	0.5	
DNA	50-100 ng	2	2	2	2.5
Total volume (µl)	..	20	24	20	5

DDW: double-distilled water; F.P. Forward Primer; R.P. Reverse Primer.

Table 5.6: Reaction Conditions for PCR Amplification for the Candidate Gene Polymorphisms.

Steps	*ACE* *Temp-Time*	*MYH7* *Temp-Time*	*MYBPC3* *Temp-Time*
Pre-denaturation	94°C - 5 mins.	94°C - 5 mins.	94°C - 5 mins.
Denaturation	94°C - 45 sec.	94°C - 30 sec.	94°C - 30 sec.
Annealing	58°C - 30 sec.	62.1°C - 30 sec.	63°C - 30 sec.
Initial Extension	72°C - 45 sec.	72°C - 30 sec.	72°C - 30 sec.
Final Extension	72°C - 4 mins.	72°C - 5 mins.	72°C - 5 mins.
PCR cycles	**40**	**36**	**40**

5.8.2 Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) is an extensively utilised genotyping method that entails breaking down genomic DNA using specific restriction

enzymes, then separating and analysing the resulting DNA fragment patterns to detect variations in sequence length or the presence of polymorphisms. A restriction enzyme recognises a specific short DNA sequence of length 4-6 bp called a recognition site and cleaves it. The cleaved fragments are then separated on an agarose gel. The restriction enzyme for this study was selected by using New England Biolabs (NEB) cutter Version 2.0 software (http://nc2.neb.com/ NEBcutter2/). The restriction enzymes used for the digestion of the selected polymorphisms are listed in Table 5.7. The reaction mixture details used for RFLP for genotyping of polymorphisms are represented in Table 5.8.

Table 5.7: Details of Restriction Enzymes Used for the Candidate Gene Polymorphisms.

Gene Polymorphism	Enzyme Used	Genomic Source	Recognition Site	Temperature Conditions
MYH7 G>A	*Taq-I* (Thermo Fisher Scientific)	*Thermus aquaticus*		Overnight at 37°C

Table 5.8: Composition of the RFLP Reaction Mixture for the Candidate Gene Polymorphisms.

Reagents	Volume(µl)
Nuclease-Free Water	7.8
Restriction Buffer	2
Restriction Enzyme (10U/ µl)	0.2
PCR Product	10
Total	**20**

5.9. TaqMan-Based Real-Time PCR Single Nucleotide Polymorphism Genotyping Assay

Genotyping was performed using allele-specific PCR and the TaqMan SNP genotyping technique. The BAG3 gene's rs2234962 variation was genotyped using the TaqMan allele discrimination test. Utilising the Bio-Rad CFX384 Touch Real-Time System (Bio-Rad Laboratories, Inc., USA), the procedure was completed. Primers and probes labelled with FAM and VIC were provided by Applied Biosystems (Thermo Fisher Scientific, Pleasanton, CA, USA) for the TaqMan SNP genotyping tests. TaqMan

master mix with Uracil-N-Glycosylase (UNG) supplied by Applied Biosystems (Thermo Fisher Scientific, Foster City, CA, USA). The assay includes two minor groove binders (mgb) probes and a forward and reverse primer that are specific to nucleotide of interest. One probe is tagged with carboxy-fluorescein (FAM), and the other is labelled with tetra chloro-fluorescein dye (VIC).

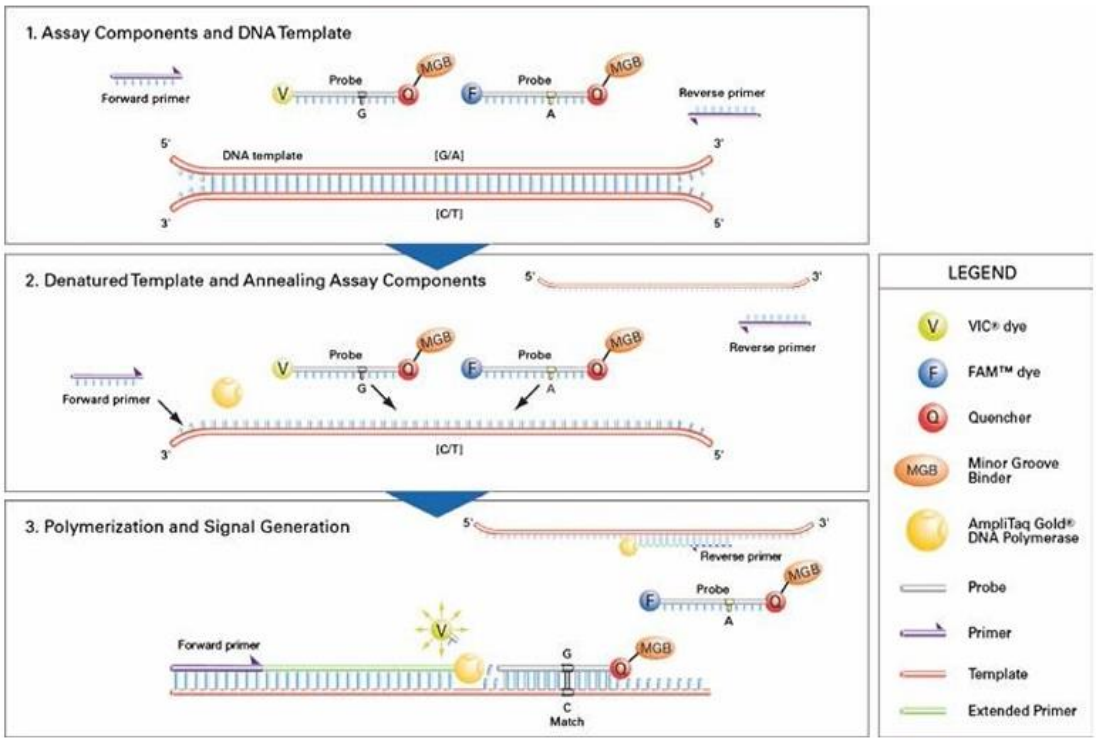


Figure 5.1: Allele Discrimination Principle in TaqMan SNP Genotyping Assays. *(1) TaqMan SNP Genotyping Assays amplify and identify polymorphic sequences using forward/reverse primers and two allele-specific, dye-labelled probes. Each probe contains a minor groove binder, a 3' non-fluorescent quencher (NFQ), and a 5' fluorescent dye for improved binding specificity. (2) Denatured template and components for the annealing test. (3) Taq polymerase finds the annealed probe while beginning to synthesise a new strand, resulting in polymerisation and signal production. It separates the 5'-bound fluorescent dye of the relevant probe (5'-3' exonuclease activity of Taq polymerase). Now that the fluorescence signal has been quenched, laser can be used to detect it.* (Schleinitz et al., 2011)

The TaqMan genotyping assay was diluted from 40x to 20x by using nuclease-free water. A total of 5µl of PCR reaction mix was created. 1.25 µl of TaqMan UNG master mix and 0.25 µl of 20x assay with a DNA volume of 2 µl and a concentration of

10 ng/µl are contained. 1.5 µl of distilled water was also added to make up the volume. The cycling conditions are 2 minutes at 50°C, 10 minutes at 95°C, then 40 cycles each of 95° C denaturation for 15 seconds, and 1 minute annealing/extension at 60°C with an end marker—were taken from the manufacturer. The Bio-Rad CFX384 Touch Real-Time system was employed to quantify fluorescence specific to each allele. Every sample was tested using 384 plate configurations. Samples with ambiguously named genotypes or those with failing genotyping were re-examined for final inclusion. One hundred randomly selected samples were re-genotyped to do the cross-validation. To monitor extraneous nucleic acid contamination, two no-template controls (NTC) were also added to each pair. Cluster plots were used to display the genotyping results. Three genotype-specific clusters are depicted in the plot. Three distinct genotype clusters are shown in the plot. The orange and blue cluster represents the homozygous genotype calls (HOMO), while the heterozygous genotype calls (HET) are represented by the green cluster, as shown in Figure 5.2.

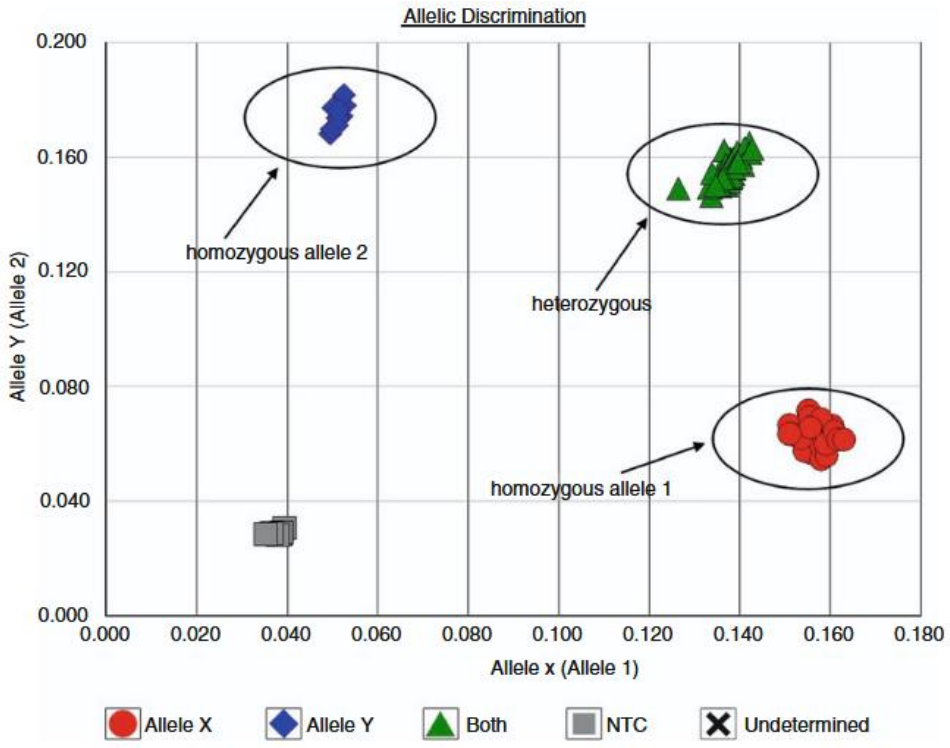


Figure 5.2: Detection of SNP allele after qPCR. *Heterozygotes that carry both alleles or homozygotes for either Allele 1 or 2 will be identified based on genotype. Non-template NTC control* (Schleinitz et al., 2011).

5.10. Conditions for Fluorescence Based qPCR Assay

The isolated DNA from blood samples by using phenol chloroform extraction method were used in this Telomere length measurement assay.

A quantitative PCR (qPCR) assay utilizing a fluorescently labeled probe was performed to detect telomere products generated by a specific set of Telomere primers, as described by Cawthon (2009). The telomere-specific probe (**TELO P**) was predesigned to be 27 bases in length, incorporating a FAM fluorophore at the 5' end and a BHQ quencher at the 3' end. For the single-copy reference gene, albumin, a predesigned probe (**ALB P**) was developed with a length of 29 bases, incorporating a HEX fluorophore at the 5' end and a BHQ quencher at the 3' end. All primers and probes used were synthesized by Applied Biosystem Thermos Fisher Scientific. This design improves the accuracy and reliability of telomere quantification while minimizing nonspecific binding commonly observed in earlier methods (Sethi et al., 2021; Cawthon, 2009). To ensure the accuracy and reproducibility of the results, all samples were analyzed in duplicate. The specific sequences of the primers and probes used for both telomere and albumin (single-copy gene) are detailed in Table 3.9

5.10.1. qPCR Conditions for Probes (TELO P and ALB P)

The 10 µl qPCR reaction mixture was finally prepared with the following components: 1X PCR master mix (Thermofisher scientific), 0.4 µM TELO F (forward primer), 0.2 µM TELO R (reverse primer), 0.3 µM TELO P probe, 0.5 µM ALB F (forward primer), 0.5 µM ALB R (reverse primer), 0.5 µM ALB P probe, (Thermofisher scientific) and 10 ng of DNA. The assay was performed using the Biorad Real-Time Polymerase Chain Reaction system. Each sample was analyzed in duplicate, totaling 200 samples processed across two independent sets of 100 cases and 100 control samples, respectively.

The optimal conditions for Quantitative Real Time PCR were established by evaluating different thermal cycling protocols. The cycling stages were structured as follows: Stage 1 included a single cycle at 95ºC for 5 minutes; Stage 2 comprised 5 cycles at 94ºC for 15 seconds followed by 49ºC for 1 minute; and Stage 3 involved 35 cycles at 85ºC for 30 seconds, 49ºC for 30 seconds, and 59ºC for 1 minute, with

fluorescence signal attainment taking place at 59ºC for both telomere and single copy gene probes.

5.10.2. Data Collection and Analyses for Telomers

Using CFX-Pro software, the dataset was initially produced in Excel format. To calculate Relative Telomere Length (RTL), duplicate measurements were averaged to get a mean Cycle threshold (Ct) values. By using the formula 2^(Ct telomeres) / 2^(Ct single copy gene) = 2^ΔCt, as explained by Schmittgen and Livak (2008), relative telomere length (RTL) was calculated based on the Cycle threshold (Ct) values. By comparing the number of telomere copies in the sample to a single-copy gene, this technique calculates the Relative Telomere Length (Montpetit et al., 2014).

Table 5.9: Telomere and Albumin (Single-Copy Gene) Primer and Probe Sequences.

S. No	Oligomer IDs	Oligomer Sequences 5'-3'
1	TELO F (F.P)	ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT
2	TELO R (R.P)	TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA
3	TELO-Probe	AAACCCAAACCCAAACCCAAACCTTAG
4	ALB F (F.P)	AAGCTGAGTTTGCAGAAGTTTC
5	ALB R (R.P)	ATATCGACGACTCTTTACCCTG
6	ALB- Probe	CACGGAATGCTGCCATGGAGATCTGCTTG

F.P-Forward Primer, R.P-Reverse Primer

5.11 Statistical Analysis

The mean and standard deviation of non-genetic variables were determined. The difference between patients and controls was determined using the student's t-test. by using Statistical Package for Social Sciences (SPSS version 25). Genotypic frequency and allelic frequency were analyzed by the gene counting method. Pearson's chi-square goodness-of-fit test was applied to assess Hardy-Weinberg Equilibrium (HWE) and to compare genotypic frequency variations between the two study groups. To evaluate the potential relationship of disease risk, odds ratios (OR) with 95% CI were calculated under different genetic models by using the SPSS version 25 software. OR for studying the association of non-genetic factors was carried out by using SPSS mentioned above

and MedCalc software (http://www.medcalc.org/calc/ odds_ratio.php). A p-value below 0.05 was considered as statistically significant value.

5.12 Power of the Study for Sample Size Determination

The power of a study refers to its ability to detect a true effect and is influenced by factors such as effect size, sample size, study design, and the predefined false-positive rate. In this study, the power was calculated using the CaTS power calculator (Skol et al., 2006) (CaTS Power Calculator). An optimal study power is generally considered to be 80%. For this analysis, a disease prevalence of 0.06% was used.

5.13 Gene-Gene Interaction Analysis

Using Multifactor Dimensionality Reduction (MDR) software (version 3.0.2), the selected polymorphisms' gene–gene interactions were detected and characterized in accordance with Martin et al. (2006) approach. MDR is especially made to identify synergistic genetic effects on a trait, even in cases when there are no discernible main effects from individual loci. We chose the genotype combinations that produced the best cross-validation consistency (CVC) and test balanced accuracy (TBA) from the suite of multi-locus models that were produced. We compared the observed TBA with its empirical distribution using a 1000-permutation test to determine statistical significance. By reviewing the interaction dendrograms generated by MDR for the best-performing models, we were able to visualize and comprehend the relationships between polymorphisms and diseases state.

CHAPTER-6
RESULTS AND OBSERVATIONS

The results and the major findings about cardiomyopathy in the study population are presented in this section. The proportion and frequency of cardiac cases are calculated by analyzing the data. Some subgroups that are compared include age, gender, and research groups (Case vs. Control). The significance of the changes that were identified was evaluated using statistical tests. The results' interpretation is supported by tables and figures.

6.1 Prevalence of Cardiomyopathy Phenotype in the Jammu Region:

In the present study, we enrolled 250 cases with cardiomyopathy phenotypes from the Superspeciality Hospital (SSH), Government Medical College, Jammu. Among them, 214 (85%) had Dilated cardiomyopathy (DCM), 28 (11%) had Hypertrophy cardiomyopathy (HCM), 2 had Restrictive cardiomyopathy (RCM), 3 had Arrhythmogenic cardiomyopathy (ARCM), and 4 had other types of cardiomyopathies (1 Peripartum cardiomyopathy (PCM) and 2 Valvular cardiomyopathy (VCM) (Figure 6.1).

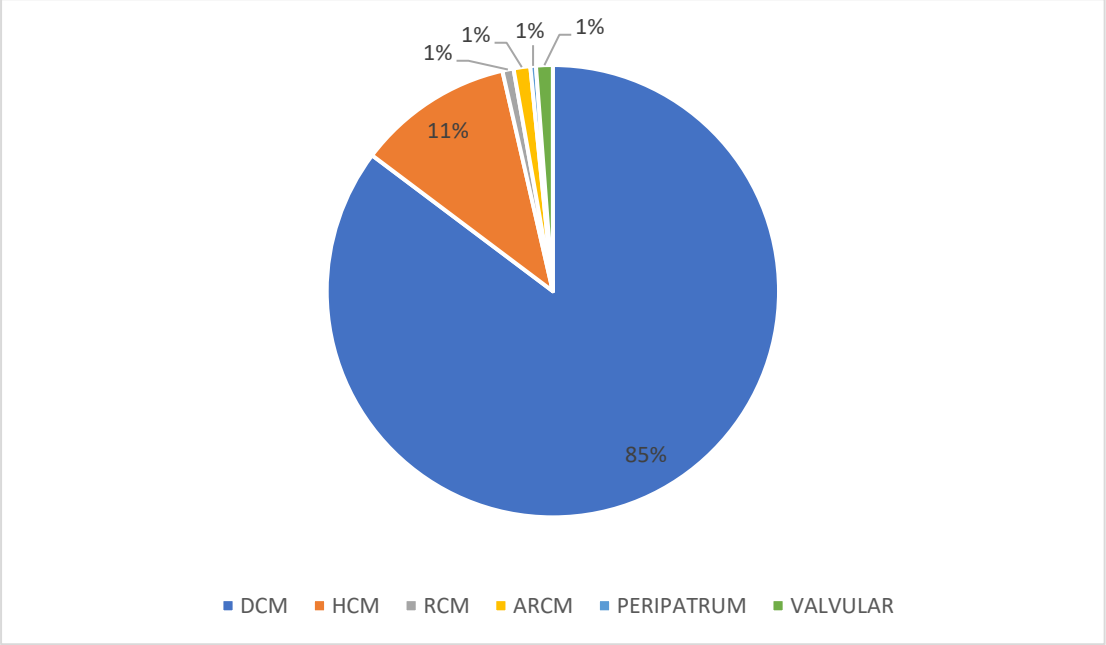


Figure 6.1: Pie chart showing the total distribution of different types of cardiomyopathies in the population of the Jammu region

As shown in Table 6.1, among the 250 cardiomyopathy cases studied in the Jammu region, 53.6% were reported shortness of breath, 45.6% were asymptomatic and only 0.8% experienced shortness of breath occasionally. A more pronounced symptom

emerged during exertion, with 84.8% experiencing shortness of breath while walking uphill, indicating significant exercise intolerance. Furthermore, chest pain or discomfort was reported by 59.2% individuals, and present occasionally in 15.2% but absent in 25.6% of the cases, suggesting considerable symptomatic heterogeneity across the cardiomyopathy phenotypes.

Table 6.1: Clinical Characteristics of Cardiomyopathy Study Participants in the Jammu Region

Symptoms **Cases (n=250)**	**Yes**	**No**	**Occasionally**
Shortness of breath	134	114	2
Shortness of breath during walk uphill	212	34	4
Chest pain or discomfort	148	64	38

6.2 Non-Genetic Risk Factors

Table 6.2 compares demographic characteristics between cardiomyopathy cases (n= 250) and healthy controls (n = 500). The average age of individuals in the case group was 57.7 ± 11.10 years, slightly higher than the control group (56.19 ± 10.05 years). Although the age difference was not statistically significant (p=0.062), it was not sufficient to indicate a meaningful age-based distinction between the groups. This suggests that they are age-matched controls.

Body weight, however, demonstrated a statistically significant difference, with cases exhibiting a higher mean weight (61.75 ± 10.61 kg) compared to controls (59.67 ± 8.92 kg), yielding a p-value of 0.008. This increase in weight among patients may reflect underlying metabolic or hemodynamic burdens associated with cardiomyopathy. Despite this weight difference, height did not significantly vary between groups (165.03 ± 5.69 cm in cases vs. 164.41 ± 9.30 cm in controls; p = 0.256), suggesting that stature was relatively uniform across the studied population.

The Body mass index (BMI), a composite metric derived from weight and height, was modestly elevated in the case group (22.63 ± 3.52 kg/m²) relative to the

control group (22.15 ± 3.35 kg/m²), but this difference was not statistically significant (p = 0.072). Nonetheless, the trend toward higher BMI in cases may hint at subtle shifts in body composition that do not reach clinical thresholds.

The most notable distinction was observed in gender distribution. The case group comprised 60.8% males (152) and 39.2% females (98), while the control group included 49.4% males (247) and 50.6% females (253). This gender-based variation was highly significant (p=0.003), indicating a potential male predisposition to cardiomyopathy in the studied cohort. These findings were further substantiated by chi-square and Fisher's exact test results (see Table 6.4), alongside odds ratio estimates indicating a higher likelihood of cardiomyopathy in males.

Overall, these findings highlight gender and body weight as statistically significant differentiators between cardiomyopathy patients and controls, whereas age, height, and BMI showed no meaningful differences in this population.

Table 6.2: Comparison of Demographic Factors Between the Case and Control Groups

Sr. no	Parameters	Case (n = 250) Mean± S.D	Control (n = 500) Mean± S.D	p-value
1.	Age (in years)	57.7±11.10	56.19±10.05	0.062
2.	Weight (kg)	61.75±10.61	59.67±8.92	0.008
3.	Height (cm)	165.03±5.69	164.41±9.30	0.256
4.	Gender (M/F)	152 / 98	247 / 253	0.003
5.	BMI (kg/m²)	22.63±3.52	22.15±3.35	0.072

Student's t-test used for the comparison between cases and control group. Statistically significant values (p < 0.05), S.D Standard Deviation

6.2.1 Comparative Analysis of Biochemical Parameters Between Cases and Controls

A comparative analysis of biochemical parameters between the disease (Case) and Control groups revealed several significant differences, highlighting distinct metabolic and physiological profiles associated with the disease condition. The biochemical parameters data were collected from patient's blood sample reports.

Cholesterol and Triglycerides

The disease group demonstrated markedly higher cholesterol levels (236.42±44.28 mg/dL) compared to the control group (152.45±40.93 mg/dL, $p < 0.001$), indicating a significantly worse lipid profile among individuals with the disease. Elevated cholesterol is a well-recognised risk factor for cardiac diseases and is often linked to atherosclerosis, hypertension, and metabolic syndrome. The substantial difference suggests that dyslipidaemia may play a key role in the disease pathology.

Similarly, triglyceride levels were significantly elevated in the disease group (222.37±54.30 mg/dL) compared to the control group (136.40±60.07 mg/dL, $p < 0.001$). Elevated triglycerides are often associated with insulin resistance, obesity, and type 2 diabetes, which are frequently observed in chronic disease conditions. The higher triglyceride levels in the disease group suggest a greater risk of metabolic and cardiovascular complications, emphasising the need for lipid management strategies in affected individuals.

High-Density Lipoprotein (HDL)

Conversely, High-Density Lipoprotein (HDL) levels, which offer protective effects against cardiovascular disease, were significantly lower in the disease group (40.93±8.38 mg/dL) compared to the control group (42.96±10.88 mg/dL, $p < 0.05$). Reduced HDL levels are indicative of a diminished capacity to clear excess cholesterol, which may contribute to plaque buildup in arteries and increased cardiovascular risk (CVD). The inverse relationship between HDL and cardiovascular risk underscores the clinical relevance of this difference, as individuals with the disease may have a higher susceptibility to atherosclerotic events.

Electrolytes: Sodium and Potassium

The disease group exhibited significantly higher sodium levels (139.83±5.90 mmol/L) compared to the control group (137.70±14.88 mmol/L, $p < 0.030$). Although the difference is modest, even minor sodium elevations can contribute to fluid retention and increased blood pressure, both of which are associated with chronic disease conditions. Elevated sodium levels may reflect dietary factors or impaired renal function commonly linked to disease states.

In terms of potassium, the disease group also had significantly higher levels (4.46±1.04 mmol/L) compared to the control group (4.174±0.641 mmol/L, $p < 0.034$). Elevated potassium may indicate electrolyte imbalances or reduced renal clearance, both of which are frequently associated with chronic kidney disease (CKD), hypertension (HT), or metabolic disorders. Higher potassium levels in the disease group could also suggest altered cellular metabolism or impaired excretion, necessitating careful monitoring of electrolyte balance.

Urea Levels

Interestingly, urea levels were significantly lower in the disease group (26.36±19.59 mg/dL) compared to the control group (35.54±32.22 mg/dL, $p < 0.001$). This finding may suggest reduced protein metabolism or altered renal function in individuals with the disease. While higher urea levels are typically indicative of renal impairment or dehydration, the lower levels in the disease group could reflect reduced muscle mass, lower protein intake, or altered renal clearance capacity. This may warrant further clinical investigation into renal function and nutritional status in the disease group.

Liver Enzymes: SGOT and SGPT

The Serum Glutamic-Oxaloacetic Transaminase (SGOT) level reveals no significant variation across the groups ($p > 0.05$), with mean values of 39.24±11.82 U/L in the disease group and 37.88±32.86 U/L in the control group. This suggests that liver function, as indicated by SGOT, remains relatively unaffected by the disease condition. In contrast, Serum Glutamic-Pyruvic Transaminase (SGPT) levels were significantly higher in the disease group (52.59±11.85 U/L) than the control group (42.25±34.45

U/L, $p < 0.001$). Elevated SGPT levels indicate liver stress or damage, which may be associated with inflammation, fatty liver disease, or metabolic syndrome. SGPT is a marker of hepatic cell injury, and its elevation in the disease group suggests potential liver involvement or underlying metabolic disturbances. This finding highlights the need for liver function monitoring and preventive interventions in individuals with the disease.

Table 6.3: Comparison of Biochemical Parameters Between the Case and Control Groups

Sr. No	Parameters	Case (n = 250) Mean±S.D	Control (n = 500) Mean±S.D	p-value
1.	Cholesterol	236.42±44.28	152.45±40.93	0.001
2.	Triglycerides	222.37±54.30	136.40±60.07	0.001
3.	High Density Lipoprotein	40.93±8.38	42.96±10.88	0.010
4.	Sodium	139.83±5.90	137.70±14.88	0.030
5.	Potassium	4.46±1.04	4.17±0.641	0.034
6.	Urea	26.36±19.59	35.54±32.22	0.001
7.	SGOT	39.24±11.82	37.88±32.86	0.525
8.	SGPT	52.59±11.85	42.25±34.45	0.001

Student's t-test used for the comparison between cases and control group. Statistically significant ($p < 0.05$) values, S.D Standard Deviation

6.2.2 Dietary and Lifestyle Factors and Their Associations with Disease

The association between cardiomyopathy status and various dietary, lifestyle, and clinical parameters was assessed using Pearson's Chi-square test (Table 6.4). Statistically highly significant associations ($p < 0.001$) were observed across all variables examined, emphasising the multifactorial nature of disease risk.

Among the behavioural and dietary factors, caffeine consumption demonstrated a strong association with disease status ($\chi^2 = 213.325$, $p < 0.001$), suggesting a moderate-to-strong effect. Tobacco use yielded the highest association ($\chi^2 = 229.044$, $p < 0.001$), followed closely by smoking behaviour ($\chi^2 = 166.458$, $p < 0.001$), identifying these exposures as critical lifestyle-linked risk factors. Alcohol intake was also strongly associated ($\chi^2 = 89.995$, $p < 0.001$), further implicating substance use in disease susceptibility. Clinically, the presence of hypertension ($\chi^2 = 141.184$, $p < 0.001$) and diabetes ($\chi^2 = 84.507$, $p < 0.001$) showed substantial associations with the disease group, reinforcing their established roles in cardiovascular pathophysiology. Among dietary practices, milk product consumption ($\chi^2 = 71.035$, $p < 0.001$) and vegetarian versus non-vegetarian diet type ($\chi^2 = 28.668$, $p < 0.001$) were also significantly associated, while oily food intake showed a more modest but significant link ($\chi^2 = 26.764$, $p < 0.001$). In addition, gender was significantly associated with disease status ($\chi^2 = 8.700$, $p < 0.003$), though the effect size was comparatively small, suggesting a minor but noteworthy demographic influence.

Overall, these findings highlight that modifiable lifestyle factors particularly caffeine, tobacco, alcohol, and dietary patterns alongside metabolic comorbidities such as hypertension and diabetes are significantly associated with cardiomyopathy risk. These insights advocate for the integration of preventive lifestyle interventions as a central strategy in disease risk mitigation and long-term management.

Table 6.4: Association Between Disease and Control Groups with Various Dietary and Lifestyle Parameters

S. No	Parameters	Characteristics	Case (n = 250)	Control (n = 500)	χ^2 (Pearson Chi-square)	p-value
1.	Caffeine	No	9	265	213.32	0.001
		Yes	199	231		
		Occasional	42	4		
2.	Oily Food	No	73	209	26.76	0.001

		Yes	89	198		
		Occasional	88	93		
3.	Milk products	No	41	10	71.03	0.001
		Yes	161	437		
		Occasional	48	53		
4.	Diet	Non - Vegetarian	145	187	28.66	0.001
		Vegetarian	105	313		
5.	Smoke	No	94	417	166.45	0.001
		Yes	140	65		
		Occasional	15	18		
6.	Alcohol	No	88	356	89.99	0.001
		Yes	126	107		
		Occasional	36	37		
7.	Tobacco	No	95	441	229.04	0.001
		Yes	129	29		
		Occasional	26	30		
8.	Hypertension	No	167	488	141.18	0.001
		Yes	82	12		
9.	Diabetes	No	210	500	84.50	0.001
		Yes	40	0		
10.	Gender	Male	152	247	8.70	0.003
		Female	98	253		

Pearson Chi-square test (χ^2) was applied between Cases and Control groups to check their association with above factors. Statistically significant values ($p < 0.05$). OR= odd ratios

6.2.3 Predictive Analysis of Risk Factors Using Binary Logistic Regression

Binary logistic regression analysis was conducted to assess the association of various dietary, lifestyle, demographic, and biochemical variables with cardiomyopathy risk (Table 6.5). Several predictors emerged as significant contributors to disease susceptibility, with clear distinctions in directionality and magnitude of effect.

Among demographic variables, male gender showed a strong association with cardiomyopathy, with males exhibiting 5.63 times greater odds of disease compared to females (OR = 5.631, p = 0.03). Although statistically significant at the 0.05 level, the elevated odds suggest a clinically meaningful trend, reinforcing earlier findings of male predominance in cardiomyopathy epidemiology.

Regarding dietary habits, caffeine consumption demonstrated one of the strongest associations in the model. Regular caffeine intake was associated with an extraordinary increase in disease odds (OR = 15,928.63, $p < 0.001$), while occasional use also conferred significant risk (OR = 212.59, $p < 0.019$). These findings suggest a potential dose-dependent relationship that warrants mechanistic validation. In contrast, milk product consumption was significantly protective (OR = 0.011, $p < 0.030$), implying a possible beneficial role of dairy intake in cardiovascular health.

Non-vegetarian diet showed a non-significant trend toward reduced disease risk (OR = 0.312, p = 0.138), and oily food intake was not significantly associated with disease. These trends, although statistically inconclusive, may still warrant further study in larger cohorts or controlled settings.

Among lifestyle exposures, tobacco use (OR = 11.81, p = 0.062) and alcohol consumption (OR = 11.16, p = 0.063) demonstrated borderline significant associations with disease status, indicating potential high-risk behaviours that merit clinical attention. Although smoking did not achieve statistical significance (p = 0.198), the elevated odds (OR = 22.97) support its inclusion in cardiomyopathy risk models.

In the realm of clinical and biochemical parameters, absence of hypertension was strongly protective (OR = 291.31, $p < 0.001$), confirming hypertension as a major comorbid risk factor. Triglyceride levels between 150–300 mg/dL were also

significantly correlated with increased disease risk (OR = 209.54, $p < 0.001$), whereas cholesterol categories showed no significant contribution.

Several biochemical markers demonstrated independent predictive value: Lower body weight (50–75 kg) was significantly associated with lower odds of disease (OR = 0.005, $p = 0.013$).

In adults, the normal range for serum potassium is 3.5–5.0 mmol/L while the normal range for serum sodium is 135–145 mmol/L (Overwyk et al., 2021). Potassium levels showed a complex association: Extreme low range was related to a significantly elevated risk. High range (5.5-7.5) showed significance (OR = 24.21, $p = 0.053$), and Extreme high potassium showed a protective effect (OR = 0.002, $p = 0.038$), suggesting a U-shaped or biphasic pattern. As frequently observed in cardiomyopathy, the patients' significantly elevated potassium levels may be the result of abnormal renal or cellular ion regulation.

Urea levels (30–45 mg/dL) were significantly predictive (OR = 35.79, $p = 0.041$). SGPT (30–60 U/L) also emerged as a strong independent predictor (OR = 263.16, $p = 0.002$).

Conversely, sodium, SGOT, high density lipoprotein (HDL) levels, and BMI categories did not display significant associations despite extreme odd ratios values in some categories, suggesting low precision or sample instability in these strata.

In summary, this model identifies gender (male), caffeine intake, triglyceride levels, hypertension, body weight, potassium balance, liver and renal function markers (SGPT and urea) as key predictors of cardiomyopathy risk. These findings reinforce the need for integrated clinical assessment and behavioural modification strategies to address both modifiable and inherent risk factors in susceptible individuals.

Table 6.5: Binary Logistic Regression Analysis Showing Association of Dietary, Lifestyle, and Biochemical Variables with Cardiomyopathy Risk.

Variable	Beta coefficient	95% C.I.	Adjusted Odd Ratio	p-value
Gender (Male)	1.728	1.131-51.886	5.631	0.03
Caffeine (No)	-	-	-	1 (Reference)
Caffeine (Yes)	9.676	98.441-3716209.9	15928.63	0
Caffeine (Occasional)	5.359	2.440-19836.770	212.589	0.019
Oily food (No)	-	-	-	1 (Reference)
Oily food (Yes)	-0.338	0.090-3.020	0.713	0.725
Oily food (Occasional)	0.869	0.315-11.634	2.385	0.362
Milk products (No)	-	-	-	1 (Reference)
Milk products (Yes)	-4.531	0.000-0.525	0.011	0.03
Milk products (Occasional)	1.226	0.264-17.450	3.409	0.274
Diet (Nonvegetarian)	-1.163	0.073-1.545	0.312	0.138
Smoke (No)	-	-	-	1 (Reference)
Smoke (Yes)	3.134	0.223-1970.995	22.967	0.198
Smoke (Occasional)	-0.816	0.004-41.083	0.442	0.737
Alcohol (No)	-	-	-	1 (Reference)
Alcohol (Yes)	2.412	0.811-110.517	11.156	0.063
Alcohol (Occasional)	1.5	0.367-44.164	4.481	0.225
Tobacco (No)	-	-	-	1 (Reference)
Tobacco (Yes)	2.469	1.079-156.627	11.806	0.062
Tobacco (Occasional)	0.549	0.110-35.286	1.731	0.712
Hypertension (Yes)	5.674	12.843-3768.969	291.307	0.00
Diabetes (Yes)	27.942	0	1.36E+12	0.995

Age (10-30 yr)	-	-	-	1 (Reference)
Age (30-50 yr)	-1.978	0.000-4.300	0.138	0.443
Age (50-70 yr)	-3.765	0.001-1.884	0.023	0.082
Age (70-90 yr)	-0.807	0.022-24.405	0.446	0.689
Cholesterol (50-150)	-	-	-	1 (Reference)
Cholesterol (150-300)	8.306	5.10E+13	4047.045	0.529
Cholesterol (Above 300)	3.824	4.99E+11	45.773	0.771
Triglycerides (50-150mg)	-	-	-	1 (Reference)
Triglycerides (150-300 mg)	5.345	12.569-3146.187	209.543	0.00
Triglycerides (Above 300 mg)	0.105	0.098-8.689	1.111	0.931
Weight (25-50 kg)	-	-	-	1 (Reference)
Weight (50-75 kg)	-5.237	0.000-0.339	0.005	0.013
Weight (75-100 kg)	-0.227	0.037-13.349	0.797	0.878
Sodium (100-135 mmol/L)	-	-	-	1 (Reference)
Sodium (135-155 mmol/L)	1.975	0.010-1714.039	7.203	0.58
Sodium (155-175 mmol/L)	0.123	0.004-371.654	1.13	0.971
Potassium (1.5-3.5 mmol/L)	-	-	-	1 (Reference)
Potassium (3.5-5.5 mmol/L)	5.708	6.658-13192.664	301.232	0.004
Potassium (5.5-7.5 mmol/L)	3.187	1.244-818.995	24.212	0.053
Potassium (7.5 mmol/L above)	-6.23	0.000-0.765	0.002	0.038
Urea (15-30 mg/dL)	-	-	-	1 (Reference)
Urea (30-45 mg/dL)	3.578	0.944-580.211	35.789	0.041
Urea (45-60 mg/dL)	1.914	0.192-69.806	6.78	0.228
SGOT (15-30 U/L)	-	-	-	1 (Reference)
SGOT (30-45 U/L)	6.399	0	601.195	1

SGOT (45-60 U/L)	6.423	0	615.595	1
SGOT (63 above U/L)	7.762	0	2350.689	1
SGPT (below 30 U/L)	-	-	-	1 (Reference)
SGPT (30-60 U/L)	5.573	4.467-3743.668	263.162	0.002
SGPT (60-90 U/L)	1.721	0.364-17.194	5.588	0.117
HDL (below 35)	-	-	-	1 (Reference)
HDL (35-70)	-16.049	0	0	0.999
HDL (70-105)	-13.791	0	0	0.999
BMI (below 18)	-	-	-	1 (Reference)
BMI (18-25, Normal)	4.338	7.19E+72	76.586	0.777
BMI (25-30, overweight)	0.655	9.82E+10	1.925	0.966
BMI (30-40, obese)	-0.453	4.12E+10	0.636	0.976

Binary Logistic Regression applied to examine the independent effect of predictors on disease risk. Statistically significant values ($p < 0.05$).

6.3 Genetic Risk Factors

6.3.1 Genetic Analysis of ACE Gene Polymorphism (rs1799752)

6.3.1.1 Genotyping of ACE Gene Polymorphism

The *ACE* polymorphism *(I/D)* is a genetic variant characterised by the presence or absence of a 287-bp Alu I repetitive sequence in the ACE gene's intron 16. This polymorphism was genotyped using a basic PCR amplification approach, this produces three different genotypes: DD, ID and II. The insertion allele (I) is the ancestral or major allele, while the deletion allele (D) is the risk or minor allele, which is associated with higher *ACE* enzyme activity. Figure 6.2 shows the PCR and electrophoresis findings, which were acquired by separating the amplified products on a 1.8% agarose gel. The II and DD genotypes are represented by bands of 490 and 190 bp, respectively, whereas the ID genotype is characterised by both 490bp and 190bp bands (Figure 6.2).

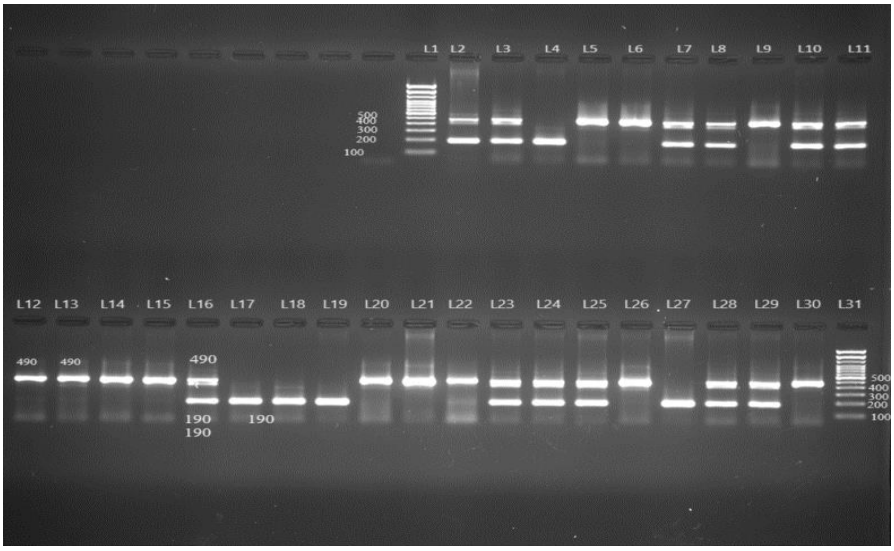


Figure 6.2: Representation of 1.8% Agarose gel image displaying the PCR product of ACE gene polymorphism. L1 and L31: 100bp ladder. L2, L3, L7, L8, L10, L11, L16, L23, L24, L25, L28, L29: 190bp, 490bp (Heterozygous type). L5, L6, L9, L12, L13, L14, L15, L20, L21, L22, L26, L30: 490bp (Homozygous wild) L4, L17, L18, L19, L27: 190bp (Homozygous mutant/variant)

6.3.1.2 Genotyping and Allelic Frequency Distribution and Association of ACE Gene Polymorphism

The genotypic and allelic frequency distribution of *ACE* polymorphism among cardiomyopathy cases and control groups is present in Table 6.6, and Hardy-Weinberg equilibrium (HWE) calculations were performed to assess genetic variation within the population, the studied population was significantly in HWE. The occurrence of the II (wild type), ID (heterozygous), and DD (variant) genotypes varied between the two groups. Among the cardiomyopathy cases, the II genotype was observed in (25.2%) individuals, the ID genotype in (48.4%) individuals, and the DD genotype in (26.4%) individuals. In contrast, the control group (n=500) showed a higher frequency of the II genotype (32.6%), a comparable frequency of ID genotype (49.0%), and a lower prevalence of DD genotype (18.4%). The allelic distribution also revealed differences between the groups. The frequency of the I (wild type) allele was 49.4% in the cardiomyopathy cases, whereas it was higher in the control group at 57.1%. Conversely, the D (risk) allele, associated with disease susceptibility, was more prevalent in cases (50.6%) compared to controls (42.9%) present in table 6.6. This suggests a possible association between the presence of the D allele and an elevated risk of cardiomyopathy.

Both populations' genetic stability was validated using Hardy-Weinberg equilibrium (HWE) calculations. With a p-value of 0.8, the case group considerably conforms to HWE, but the control group adheres even more strongly (p-value of 1). The equilibrium in both groups suggests that there is no significant selection pressure or population stratification affecting the distribution of *ACE* polymorphisms. Overall, the findings highlight that the D allele and DD genotype are more prevalent in cardiomyopathy cases, suggesting a potential genetic predisposition to the disease.

Table 6.6: Showing Genotypic and Allelic Frequency Distribution of *ACE* Polymorphism Among Cases and Controls Along with χ2 and p-values for Hardy-Weinberg Equilibrium Calculations.

Category	Genotypes Frequency (%)			Alleles Frequency (%)		χ^2 Chie square	p-value
	II (Homozygous wild type)	ID (Heterozygous type)	DD (Heterozygous mutant)	I (Wild)	D (Risk)		
Cases (n=250)	63 (25.2%)	121 (48.4%)	66 (26.4%)	0.494 (49.4%)	0.506 (50.6%)	0.3965	0.8
Controls (n=500)	163 (32.6%)	245 (49.0)	92 (18.4%)	0.571 (57.1%)	0.429 (42.9%)	0.0001	1.0

HWE- Hardy Weinberg Equilibrium p values. Significant values ($p<0.05$)

6.3.1.3 Association of ACE Gene Polymorphism and Cardiomyopathy Under Different Genetic Models

The logistic regression analysis for *ACE* polymorphism was conducted to evaluate its potential association with cardiomyopathy under different genetic models, including co-dominant, dominant, recessive, and allelic models. The results are presented in Table 6.7, with their odds-ratios (OR) and 95% confidence intervals (CI) indicating the strength of association between specific genotypes or alleles and the risk of developing cardiomyopathy.

In the co-dominant model, individuals with the ID genotype exhibited a non-significant increase in cardiomyopathy risk compared to the II genotype, with an OR of 1.27 (95% CI: 0.89–1.83; p = 0.1860). However, the DD genotype was significantly associated with an elevated risk of cardiomyopathy, showing an OR of 1.85 (95% CI: 1.21–2.85; p = 0.0048), indicating that individuals carrying the homozygous variant

genotype have nearly twice the risk of developing cardiomyopathy compared to those with the wild-type II genotype. In the dominant model, where both ID and DD genotypes were combined and compared against the II genotype, the risk of cardiomyopathy was elevated significantly (OR = 1.43, 95% CI: 1.02–2.02; p = 0.0378). This suggests that carrying at least one copy of the D allele contributes to increased susceptibility to cardiomyopathy.

The recessive model, which compares the DD genotype against the combined ID and II genotypes, also demonstrated a significant association with cardiomyopathy risk. Individuals with the DD genotype had a 1.59 times higher risk of developing the disease (OR = 1.59, 95% CI: 1.10–2.28; p = 0.0117), further supporting the role of the homozygous D allele in disease predisposition.

In the allelic model, the D allele was found to be significantly associated with an increased risk of cardiomyopathy. Individuals carrying the D allele had a 1.36-fold higher risk of developing the disease compared to those carrying the I allele (OR = 1.36, 95% CI: 1.09–1.69; p = 0.0048). This indicates that the presence of the D allele, regardless of genotype, contributes to cardiomyopathy susceptibility.

Overall, the logistic regression analysis suggests that the DD genotype and the D allele are significantly associated with an increased risk of cardiomyopathy, highlighting a potential genetic predisposition. The dominant and recessive models further support the impact of the D allele in disease development, with the strongest association observed in individuals homozygous for the risk allele (DD genotype). These findings underscore the role of ACE polymorphism in cardiomyopathy pathogenesis and suggest that genetic screening for the D allele could be useful in identifying at-risk individuals.

Table 6.7: Showing Logistic Regression Analysis for ACE Gene Polymorphism

Genetic Models	Genotypes & Alleles	Cardiomyopathy Cases (n=250)	Controls (n=500)	OR (95% CI)	p-value
Co-dominant	II	63	163	1 (Reference)	-
	ID	121	245	1.27 [0.89 - 1.83]	0.1860
	DD	66	92	1.85 [1.21 - 2.85]	0.0048
Dominant	ID+DD	187	337	1.43 [1.02 - 2.02]	0.0378
	II	63	163	1 (Reference)	-
Recessive	DD	66	92	1.59 [1.10 - 2.28]	0.0117
	ID+II	184	408	1 (Reference)	-
Allelic	D	253	429	1.36 [1.09 to 1.69]	0.0048
	I	247	571	1 (Reference)	-

Odd ratios calculated and p values were calculated by grouping genotypes and by binary logistic regression between Cases and Controls. Allelic comparisons were assessed by Chi-square test. Statistically significant values ($p < 0.05$) OR- odd ratios

6.3.2 Genetic Analysis of MYH7 Gene Polymorphism (rs397516208)

6.3.2.1 Genotyping of Taq1-MYH7 Gene Polymorphism

The genotyping of *MYH7* was done by using Polymerase chain reaction-based restriction fragment length polymorphism (PCR- RFLP) techniques, and the results are presented in Table 6.8. In this polymorphism, there is a single nucleotide polymorphism in which a guanine is replaced by adenine at position 4276. The *Taq-1* restriction enzyme is used for the digestion, which cuts the amplified product at the specific recognition site 5'-T↓CGA-3'. The PCR amplification produced a 299bp product, exhibited on 1.5% agarose gel Figure 6.3, which was then digested to identify different genotypes: homozygous wild (GG) type, heterozygous (GA), and homozygous variant (AA) type.

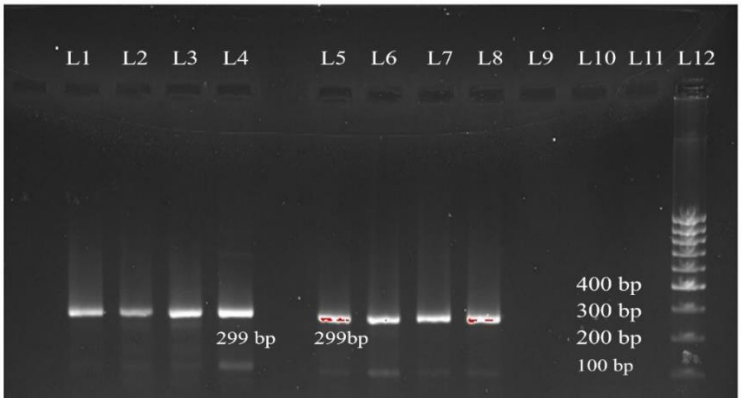


Figure 6.3: Representation of 1.5% Agarose gel image displaying the PCR product of *MYH7 G>A* polymorphism, L1-L8 has 299 bp, L11-12: empty wells and L12: 100 bp ladder

For individuals carrying the homozygous wild-type genotype (GG), the digestion resulted in three distinct fragments of 140bp, 87bp, and 72bp, indicating the presence of wild alleles on 3% agarose gel (Figure 6.4B). In contrast, the heterozygous genotype (GA) exhibited fragments of 212bp, 140bp, 87bp, and 72bp, demonstrating the presence of both the wild-type and mutant alleles (Figure 6.4A and 6.4B). The presence of the 212 bp fragment in heterozygous individuals suggests partial digestion due to the presence of one mutated allele, while the smaller fragments correspond to the uncut wild-type allele.

Individuals with the homozygous variant genotype (AA) showed only two fragments, 212 bp and 87 bp, indicating complete mutation of both alleles (Figure 6.6), which prevents full restriction enzyme cleavage at the wild-type recognition site. The absence of the 140 bp fragment in the AA genotype confirms the loss of the normal restriction site due to the polymorphic variation.

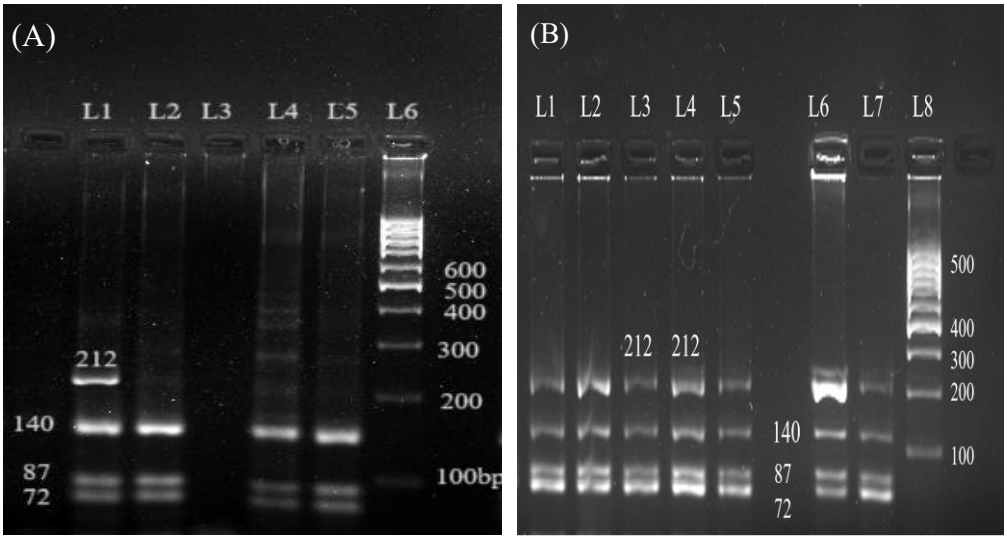


Figure 6.4A and 6.4B: Representation of 3% Agarose gel image displaying the restriction digestion product of MYH7 G>A polymorphism. **6.4A:** L 1: 212 bp,140 bp,87 bp,72 bp (Heterogenous mutant type); L 2, L 4, L 5: 140bp, 87bp, 72 bp (Homozygous wild type) L6: 100 bp ladder. **6.4B**: L1-L7: 212bp, 140 bp, 87 bp and 72 bp (Heterozygous mutant type) and L8: 100 bp ladder

The restriction profile highlights the distinct fragment patterns for different *MYH7* genotypes, enabling clear differentiation between wild-type, heterozygous, and mutant genotypes. This analysis is essential for determining the genetic variations within the MYH7 gene, which is implicated in various cardiomyopathies and other cardiac disorders.

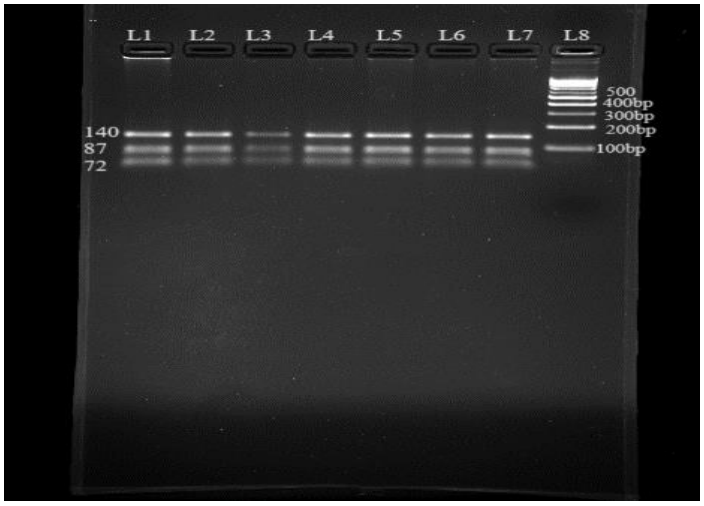


Figure 6.5: Representation of 3% Agarose gel image displaying the restriction digestion product of MYH7 G>A polymorphism L1-L7 has 140bp, 87bp,72 bp (Homozygous wild type) and L8 has 100bp ladder

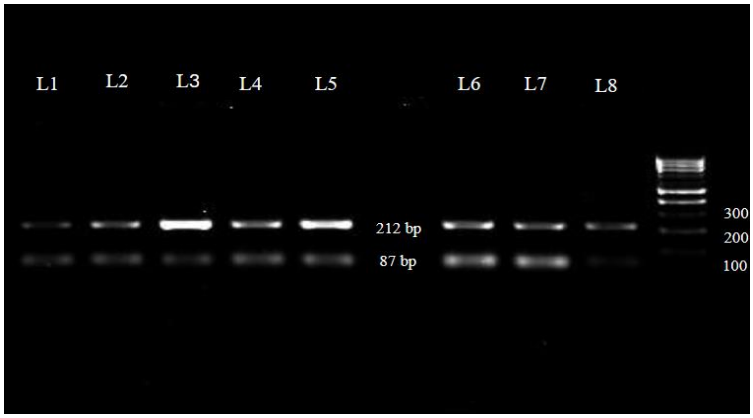


Figure 6.6: Representation of 3% Agarose gel electrophoresis image of restriction fragment polymorphism of *MYH7 G>A* L1-L8, which has 212bp and 87bp (Homozygous mutant) with a 100bp ladder

Table 6.8: Restriction Profile of MYH7 Gene Polymorphism

PCR product	Homozygous Wild type (GG)	Heterozygous type (GA)	Homozygous variant type (AA)
299	140bp 87bp 72bp	212bp 140bp 87bp 72bp	212bp 87bp
Figure 6.3	Figure 6.5	Figure 6.4A& 6.4B	Figure 6.6

6.3.2.2 Genotyping and Allelic Frequency Distribution of MYH7 G>A Gene Polymorphism

The genotypic and allelic frequency prevalence of the *MYH7* polymorphism among cardiomyopathy cases and control group is presented in Table 6.9. Among the 250 cardiomyopathy cases, the GG (wild type) genotype was observed in 46% individuals (115), the GA (heterozygous) genotype in 42.5% individuals (105), and the AA (variant) genotype in 12% individuals (30). In contrast, among the 500 control individuals, the GG genotype was found in 300 individuals (60%), the GA genotype in 174 individuals (34.8%), and the AA genotype in 26 individuals (5.2%).

In terms of allele frequency, the G (wild) allele was more prevalent in both groups, constituting 67% in cases and 77% in controls, while the A (risk) allele was

present in 33% of cases and 23% of controls. A chi-square (χ^2) test was conducted to assess deviations from Hardy-Weinberg equilibrium (HWE), yielding a χ^2 value of 0.6301 (p = 0.73) for cases, indicating that the genotype distribution was in equilibrium. However, in controls, χ^2 value was 9.2515 (p=0.99), suggesting no significant deviation from Hardy-Weinberg equilibrium.

The observed differences in genotype and allele distribution suggest a potential association between the *MYH7* polymorphism and cardiomyopathy, with a higher frequency of the A allele in cases compared to controls.

Table 6.9: Showing Genotypic and Allelic Frequency Distribution of *MYH7* Polymorphism Among Cases and Controls Along with χ2 and p-values for Hardy-Weinberg Equilibrium.

Category	Genotypes Frequency (%)			Allele Frequency (%)		χ^2 Chie-Square	p-value
	GG (Homozygous wild)	GA (Heterozygous type)	AA (Homozygous mutant)	G (Wild)	A (Risk)		
Cases (n=250)	115 (46%)	105 (42%)	30 (12%)	0.67 (67%)	0.33 (33%)	0.6301	0.73
Controls (n=500)	300 (60%)	174 (34.8%)	26 (5.2%)	0.77 (77%)	0.23 (23%)	9.2515	0.99

Hardy Weinberg p values. Significant values (p<0.05)

6.3.2.3 Association of *MYH7* Polymorphism and Cardiomyopathy Under Different Genetic Models

Table 6.10 presents the logistic regression analysis for MYH7 gene polymorphism, assessing the association of different genetic models with cardiomyopathy risk. The analysis includes co-dominant, dominant, recessive, and allelic models, with odds ratios (OR), confidence intervals (CI), and p-values indicating statistical significance.

In the co-dominant model, the GG (homozygous wild type) genotype serves as the reference category. Compared to GG, individuals with the GA (heterozygous) genotype had a significantly higher risk of cardiomyopathy (OR = 1.57, 95% CI = 1.13–2.39, p = 0.006), while those with the AA (homozygous variant type) genotype showed an even stronger association with cardiomyopathy (OR = 3.01, 95% CI = 1.70–5.30, p

= 0.0001), suggesting a dose-dependent effect of the A allele. The dominant model (GA + AA vs. GG) further supports this association, as individuals carrying at least one A allele (GA or AA) had a significantly increased risk of cardiomyopathy (OR = 1.76, 95% CI = 1.29–2.39, p = 0.0003) compared to GG carriers. This implies that even a single copy of the variant allele (A) contributes to disease susceptibility.

Similarly, in the recessive model (AA vs. GA + GG), individuals with the AA genotype had more than twice the risk of developing cardiomyopathy (OR = 2.48, 95% CI = 1.43–4.30, p = 0.0011) compared to those with at least one G allele (GA or GG). This suggests that the presence of two copies of the A allele significantly increases disease risk.

In the allelic model, the frequency of the A allele was compared with the G allele, revealing a significant association with cardiomyopathy. The presence of the A allele increased the odds of disease by 1.68 times (OR = 1.68, 95% CI = 1.32–2.14, p < 0.0001), further reinforcing its potential role as a risk allele.

Overall, these findings suggest that the A allele of the *MYH7* polymorphism is strongly associated with an increased risk of cardiomyopathy, with both heterozygous (GA) and homozygous mutant/variant (AA) genotypes contributing to disease susceptibility. The data focus on the importance of *MYH7* genetic variation in cardiomyopathy pathogenesis, warranting further investigation into its functional implications.

Table 6.10: Showing Logistic Regression Analysis for MYH7 Gene Polymorphism

Genetic Models	Genotypes & Alleles	Cardiomyopathy Cases (n=250)	Controls (n=500)	OR (95% CI)	p-value
Co-dominant	GG	115	300	1 (Reference)	
	GA	105	174	1.57 [1.13-2.39]	0.006
	AA	30	26	3.01 [1.70-5.30]	0.0001
Dominant	GA+AA	135	200	1.76 [1.29-2.39]	0.0003
	GG	115	300	1 (Reference)	
Recessive	AA	30	26	2.48 [1.43-4.30]	0.0011
	GA+GG	220	474	1 (Reference)	
Allelic	A	165	226	1.68 [1.32-2.14]	0.0001
	G	335	774	1 (Reference)	

Odd ratios calculated and p values were calculated by grouping genotypes and by binary logistic regression between Cases and Controls. Allelic comparisons were assessed by Chi-square test. Statistically significant at $p < 0.05$, OD-odd ratios

6.3.2.4 Genetic Analysis of MYBPC3 Gene Polymorphism (rs36212066)

6.3.2.5 Genotyping of MYBPC3 25bp Gene Polymorphism

The genotyping of MYBPC3 gene was done by using PCR amplification. The target DNA was amplified using certain oligonucleotide primers, and the amplified DNA region was then analysed for genotyping. To ascertain whether a deletion occurred, a 50-bp DNA ladder was used to compare the amplification results. The genetic variants were identified by the presence or absence of specific DNA fragments associated with the deletion on 3% agarose gel, as shown in Figure 6.7.

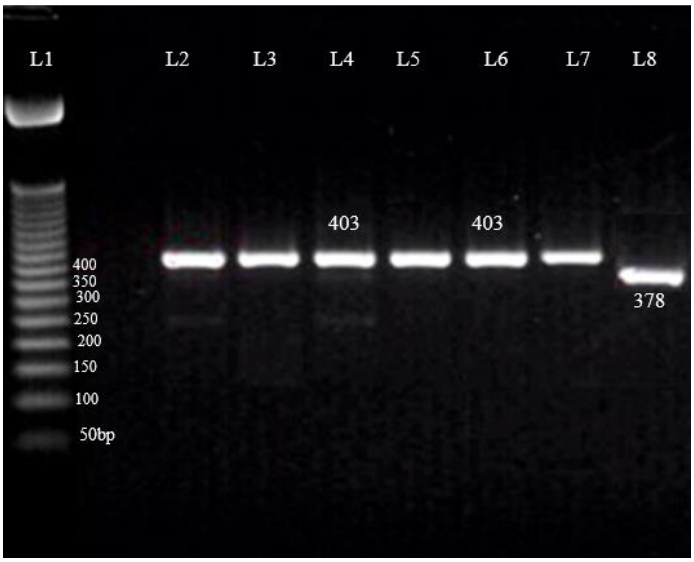


Figure 6.7: Representation of 3% Agarose gel electrophoresis of PCR product of MYBPC3 gene polymorphism. L1: 50 bp ladder and L2-L7: 403 bp (wild type) and L8: 378 bp (mutant type)

6.3.2.6 Genotyping and Allelic Frequency Distribution of MYBPC3 25bp Gene Polymorphism

Table 6.11 presents the distribution of *MYBPC3* polymorphism among cardiomyopathy cases and controls, along with the odds ratio (OR) and p-value assessing statistical significance. The data compares the WW (homozygous wild type) genotype with the DD (homozygous variant) genotype to evaluate its association with cardiomyopathy. Among cardiomyopathy cases (n = 250), 71.2% (178) individuals carried the WW genotype, whereas 28.8% (72) individuals had the DD genotype. In contrast, among controls (n = 500), 94.8% (474) individuals had the WW genotype, while only 26 individuals (5.2%) carried the DD genotype. The odds ratio (OR = 7.374,

95% CI = 4.56–11.92, p < 0.0001) indicates that individuals carrying the DD genotype have a significantly higher risk of developing cardiomyopathy, with over sevenfold increased odds compared to those with the WW genotype. But the population deviates significantly from HWE because, in this genotyping, no heterozygous genotype is found in both cases and controls. The statistically significant p-value (<0.0001) confirms the strong association between the MYBPC3 polymorphism and cardiomyopathy, suggesting that the DD genotype may play a crucial role in disease susceptibility. These findings highlight the importance of genetic screening for MYBPC3 variants in assessing the risk of cardiomyopathy and understanding its genetic basis.

Table 6.11: Showing Genotypic and Allelic Frequency Distribution of *MYBPC3* Polymorphism Among Cases and Controls Along with p-values.

Category	Genotypes Frequency (%)		Allele Frequency (%)		OR (95% CI)	p-value
	WW (Homozygous wild type)	DD (Homozygous variant)	W (Wild Allele)	D (Risk Allele)		
Cardiomyopathy Cases (n=250)	178 (71.2%)	72 (28.8%)	0.71 (71%)	0.29(29%)	7.374 [4.56-11.92]	0.0001
Controls (n=500)	474 (91.8%)	26 (5.2%)	0.948 (94.8%)	0.052 (5.2%)		

Fisher exact t test applied to check whether allele frequencies differ significantly between cases and controls. Statistically significant at $p < 0.05$. OR- odd ratios

6.3.3 Genetic Analysis of BAG 3 T>C Gene Polymorphism (rs2234962)

6.3.3.1 Genotyping of BAG3 Gene Polymorphisms (TaqMan Genotyping Assay)

The BAG3 T>C polymorphism was genotyped using quantitative PCR (qPCR) with fluorescent probes unique to each allele. Alleles C and T were tagged with VIC and FAM, respectively, in this experiment. All samples showed effective target sequence amplification in the amplification plot, showing dependable PCR performance (Figure 6.8). Additionally, based on fluorescence intensity, the allelic discrimination plot clearly distinguished between the three potential genotypes: heterozygous TC samples appeared in the intermediate zone, displaying signals for both dyes (green colour), homozygous TT samples clustered in the FAM-dominant region (blue colour), and homozygous CC samples appeared in the VIC-dominant region

(orange colour) (Figure 6.9). These findings support the qPCR assay's ability to precisely identify BAG3 gene genotypes in the population under study.

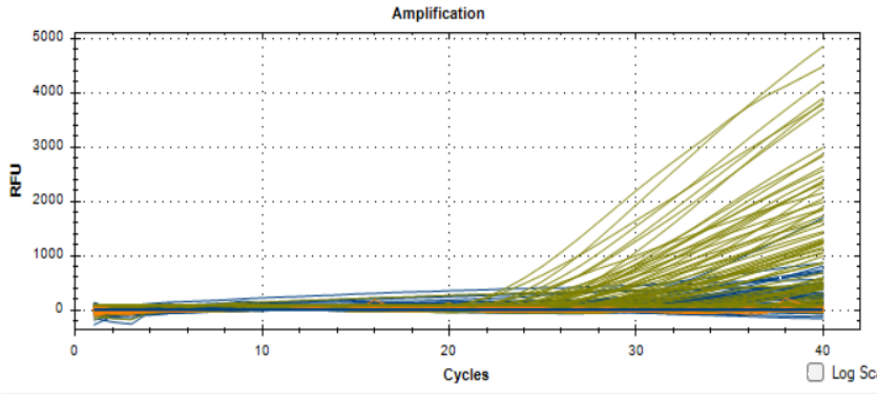


Figure 6.8: Amplification plot of BAG-3 gene generated by qPCR

6.3.3.2 Genotypic and Allelic Frequency Distribution and Association of BAG3 Gene Polymorphism

The genotypic and allelic frequency distribution of the BAG3 polymorphism among cardiomyopathy cases and controls is summarised in Table 6.12. Among the cases (n = 250), the frequencies of the TT (wild type), TC (heterozygous), and CC (variant) genotypes were 37.6% (94), 44.4% (111), and 18.0% (45), respectively, whereas among the controls (n = 500), the corresponding genotype counts were 54.0% (270), 40.0% (200), and 6.0% (30). The T allele frequency was observed to be 59.8% in cases and 74.0% in controls, while the C allele frequency was higher among cases (40.2%) compared to controls (26.0%). Hardy-Weinberg equilibrium analysis showed no significant deviation among either cases ($\chi^2 = 1.46$, $p = 0.52$) or controls ($\chi^2 = 0.78$, $p = 0.67$), indicating that the genotype distributions were consistent with expected proportions.

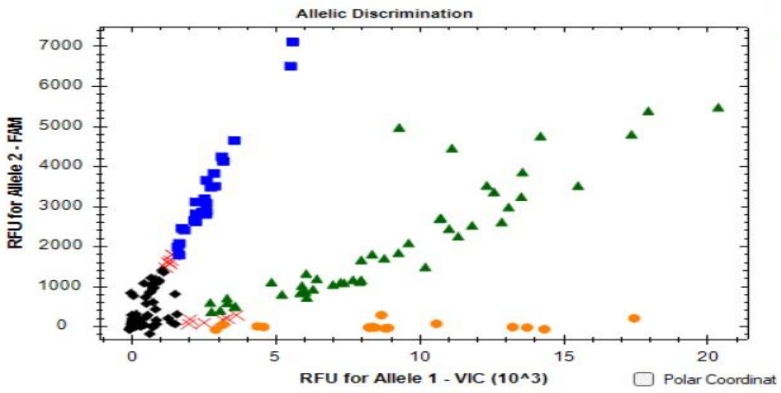


Figure 6.9: Allelic discrimination plot of BAG3-3 gene generated by qPCR

Table 6.12: Showing Genotypic and Allelic Frequency Distribution of *BAG3* Polymorphism Among Cases and Controls Along with χ2 and p-values for Hardy-Weinberg Equilibrium.

Category	Genotypes Frequency (%)			Allele Frequency (%)		χ^2	*p-value*
	TT (Homozygous wild)	**TC (Heterozygous type)**	**CC (Homozygous mutant type)**	**T (Wild)**	**C (Risk)**		
Cases (n=250)	94 (37.6%)	111 (44.4%)	45 (18%)	0.598 (59.8%)	0.4.2 (40.2%)	1.46	0.52
Controls (n=500)	270 (54%)	200 (40%)	30 (6%)	0.74 (74%)	0.26 (26.0%)	0.78	0.67

6.3.3.3 Association of BAG3 Polymorphism and Cardiomyopathy Under Different Genetic Models

Logistic regression analysis further revealed a significant association of BAG3 polymorphism with cardiomyopathy risk (Table 6.13). In the co-dominant model, individuals with the TC genotype had a 1.59-fold increased risk (OR = 1.59, 95% CI: 1.14–2.21, p = 0.005), and those with the TT genotype exhibited a markedly higher 4.30-fold increased risk (OR = 4.30, 95% CI: 2.56–7.23, p = 0.0001) compared to the CC genotype. Under the dominant model (TC + CC vs TT), the combined presence of TC and CC genotypes was associated with a 1.94-fold elevated risk (OR = 1.94, 95% CI: 1.42–2.65, p = 0.0001). Similarly, in the recessive model (CC vs TC + TT), the CC genotype conferred a 3.43-fold increased susceptibility to cardiomyopathy (OR = 3.43, 95% CI: 2.10–5.61, p = 0.0001). Allelic analysis demonstrated that carriers of the C allele had a 1.91-fold higher risk compared to T allele carriers (OR = 1.91, 95% CI: 1.52–2.40, p < 0.0001).

Table 6.13: Showing Logistic Regression Analysis for BAG 3 Gene Polymorphism

Genetic Model	Genotypes & Alleles	Cardiomyopathy Cases (n=250)	Controls (n=500)	OR (95% CI)	p-value
Co-dominant	TT	94	270	1 (Reference)	
	TC	111	200	1.59 [1.14-2.21]	0.005
	CC	45	30	4.30 [2.56-7.23]	0.0001
Dominant	TC+CC	156	230	1.94 [1.42-2.65]	0.0001
	TT	94	270	1 (Reference)	
Recessive	CC	45	30	3.43 [2.10-5.61]	0.0001
	TC+TT	205	470	1 (Reference)	
Allelic	C	201	260	1.91 [1.52-2.40]	<0.0001
	T	299	740	1 (Reference)	

Odd ratios calculated and p values were calculated by grouping genotypes and by binary logistic regression between Cases and Controls. Allelic comparisons were assessed by Chi-square test. Statistically significant values ($p < 0.05$), OR- odd ratios

6.4 Quantitative Analysis of Telomere Length in Cardiomyopathy Patient DNA Samples by qPCR to Establish its Role in Disease Predisposition

The amplification plots produced by the qPCR present in Figure 6.10 and Figure 6.11. The use of separate probes and fluorescent dyes in this experiment allows the telomere and single-copy gene products to be clearly distinguished, regardless of each other's amplification. As verified by gel electrophoresis, the qPCR results for the telomere (79 bp) and single-copy gene (116 bp) are displayed in Figure 6.12 and Figure 6.13, respectively.

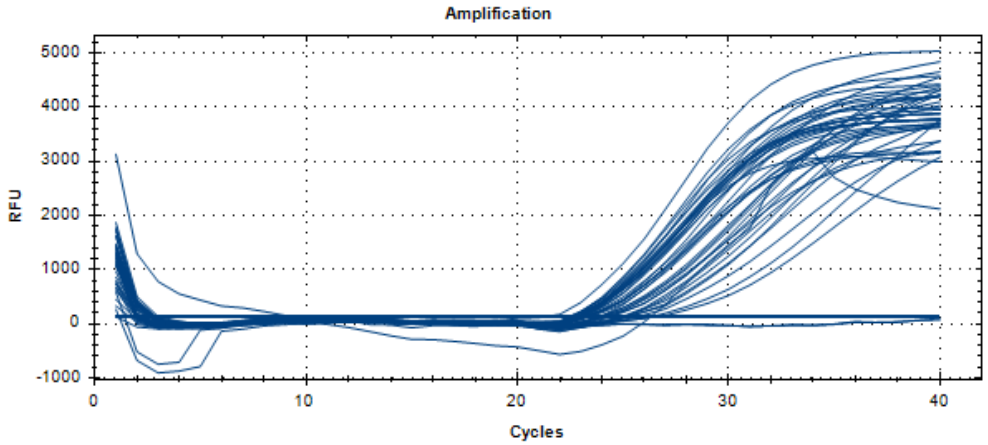


Figure 6.10: Amplification Plot of Telomere generated by qPCR

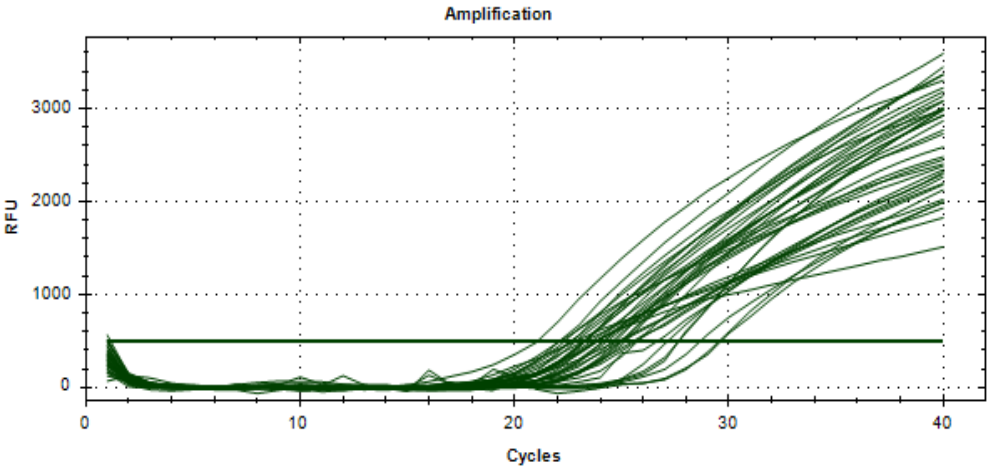


Figure 6.11: Amplification Plot of Albumin (single copy gene) generated by qPCR

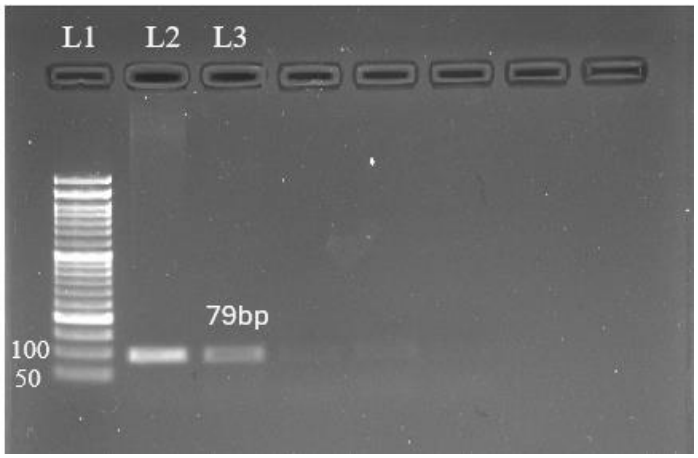


Figure 6.12: Representation on 1.5% Gel electrophoresis of PCR product of Telomere gene product. L1 is 50bp ladder and L2-L4 have 79bp product

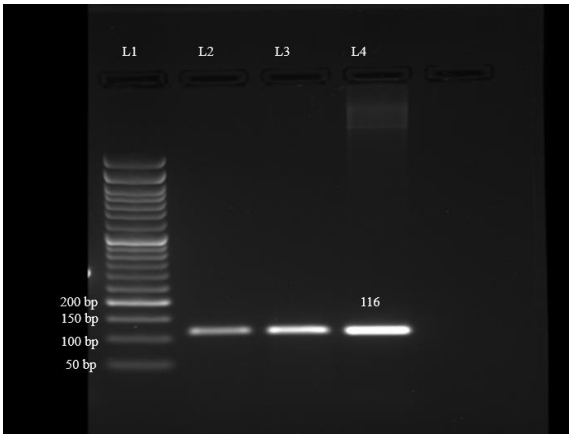


Figure 6.13: Representation on 1.5% Gel electrophoresis of PCR product of Albumin gene product. L1 is 50bp ladder and L2-L4 have 116bp product

6.4.1 Comparison of Telomere Length in Population from Jammu Region

A subgroup analysis of telomere length among different age, gender, and BMI categories revealed significant patterns across both cases and controls in Table 6.14. Individuals below 35 years exhibited significantly longer telomeres compared to those above 55 years in both groups, with telomere length declining from 1.20 to 0.95 in cases ($p = 0.03$) and from 1.50 to 1.25 in controls ($p = 0.01$), indicating an age-associated reduction in telomere length that was more pronounced in patients. When comparing between groups, telomere length was consistently shorter in cases across all age categories, including <35 years ($p = 0.002$), 35–55 years ($p = 0.05$), and >55 years ($p = 0.04$), suggesting accelerated telomere attrition in cardiomyopathy.

Gender-wise analysis showed that females had longer telomeres compared to males in both cases and controls, with statistically significant differences ($p = 0.003$ and $p = 0.020$, respectively). Similarly, BMI was inversely associated with Telomere length. Individuals with a normal BMI (<25) exhibited significantly longer telomeres than overweight/obese individuals (BMI ≥25) in both cases ($p = 0.005$) and controls ($p = 0.001$), indicating that higher BMI may contribute to Telomere shortening, possibly due to increased oxidative stress and chronic inflammation.

Collectively, these findings highlight that Telomere length is significantly influenced by age, gender, and BMI, and is markedly reduced in patients with cardiomyopathy across all demographic strata. This underscores the potential of Telomere length as a

non-genetic marker of cellular senescence, physiological aging and disease progression in the studied population.

Table 6.14: Comparison of Telomere Length in Jammu Region Population Group Subset

S. No	Parameter	(Cases) Mean±S.D	(Controls) Mean±S.D	p-value
1	Telomere Length in individuals below 35 years	1.20 ± 0.50	1.50 ± 0.55	0.002
2	Telomere Length in individuals 35-55 years	1.05 ± 0.60	1.35 ± 0.65	0.05
3	Telomere Length in individuals above 55 years	0.95 ± 0.45	1.25 ± 0.50	0.04
4	Comparison of TL between age groups <35 and >55 in Cases	1.20/0.95 ± 0.50/0.45	---	0.03
5	Comparison of TL between age groups <35 and >55 in Controls	---	1.50/1.25 ± 0.55/0.50	0.01
6	Telomere Length in Males	1.00 ± 0.50	1.30 ± 0.60	0.003
7	Telomere Length in Females	1.15 ± 0.55	1.45 ± 0.58	0.020
8	Comparison of TL: Male vs Female (within Cases and Controls)	1.00/ 1.15 ± 0.50 / 0.55	1.30/ 1.45 ± 0.60 / 0.58	0.020
9	TL in BMI < 25 (Normal)	1.30 ± 0.50	1.55 ± 0.55	0.005
10	TL in BMI ≥ 25 (Overweight/Obese)	0.95 ± 0.45	1.20 ± 0.50	0.001
11	Comparison of TL: BMI <25 vs ≥25	1.30/ 0.95 ± 0.50 / 0.45	1.55/ 1.20 ± 0.55 / 0.50	0.005

Statistically significant values ($p < 0.05$), S.D- Standard Deviations

Binary logistic regression analysis was performed to evaluate the predictive role of telomere length in relation to cardiomyopathy. In the unadjusted model (Model 1), Telomere length showed a significant inverse association with cardiomyopathy, with a beta coefficient of –0.70 ($p = 0.001$), indicating that shorter telomeres were correlated with elevated odds of having the illness. When the model was adjusted for age and gender (Model 2), the association remained significant and slightly strengthened ($\beta = –0.74$, $p = 0.00046$), suggesting that the relationship between telomere length and disease status was independent of age and gender.

Further adjustment for BMI in Model 3 maintained the statistical significance of telomere length as a predictor ($\beta = -0.65$, $p = 0.002$), confirming that the association was robust even when accounting for body mass index, as shown in Table 6.15. Overall, these findings collectively demonstrate that shorter telomere length is an independent and significant predictor of cardiomyopathy, irrespective of demographic and anthropometric confounders, and may serve as a valuable molecular biomarker for disease risk assessment.

Table 6.15: Binary Logistic Regression Analysis of Telomere Length as a Predictor of Cardiomyopathy

Model	Variables Adjusted	Beta Coefficient	S.E.	p-value
1	Telomere Length (Unadjusted)	-0.70	0.208	0.001
2	Telomere + Age + Gender (Adjusted)	-0.74	0.211	0.00046
3	Telomere + Age + Gender + BMI (Adjusted)	-0.65	0.213	0.002

Statistically significant values ($p < 0.05$), S.E. (Standard Error)

Among the non-genetic parameters, telomere length showed a significant inverse correlation with triglycerides ($r = -0.260$, $p = 0.01$), SGPT ($r = -0.188$, $p = 0.04$), and borderline significance with BMI ($r = -0.194$, $p = 0.05$). Cholesterol and SGOT demonstrated moderate negative trends. HDL showed a weak, non-significant positive correlation. Other biochemical markers, including sodium, potassium, urea, and height, did not show statistically significant associations. These findings support the role of metabolic dysfunction, particularly lipid abnormalities and liver enzyme elevation, in telomere attrition among cardiomyopathy patients (Table 6.16).

Table 6.16: Correlation Analysis of Telomere Length with Non-genetic Factors in Cardiomyopathy

Non-Genetic Factor	Pearson Correlation Coefficient (r)	p-value	Interpretation
Age	–0.142	0.09	Weak negative, not statistically significant
Height	–0.070	0.42	Very weak, not significant
BMI	–0.194	0.05*	Borderline significant, inverse correlation
Cholesterol	–0.210	0.04*	Significant inverse correlation
Triglycerides	–0.260	**0.01***	**Strong, significant inverse correlation**
High-Density Lipoprotein	+0.160	0.08	Positive trend, not significant
Sodium	–0.130	0.11	Mild inverse trend
Potassium	–0.180	0.03*	Significant inverse correlation
Urea	+0.120	0.15	Weak positive, not significant
SGOT	–0.070	0.42	Very weak inverse, not significant
SGPT	–0.230	**0.02***	**Moderate, significant inverse correlation**

Pearson Correlation Test used between Telomere Length and Non-genetic factors. Statistically significant values ($p < 0.05$)

6.5 Gene -Gene Interaction Analysis

The multidimensional reduction model is an effective technique for identifying genotypic combinations at high and low risk to forecast an increase or decrease in risk for complicated illnesses. The multifaceted data is reduced to a single dimension by using Multifactor Dimensionality Reduction (MDR) to detect and describe high-order gene-gene interactions affecting the development of cardiomyopathy disease (CM) in both the case and control groups. Table 6.17 lists the genetic markers that were used in this investigation. A dendrogram representing the outcomes of the MDR interaction analysis including all four of the examined gene polymorphisms is presented in Figure

30. Whether interactions were repetitive or synergistic within the dendrogram was determined using a color-coded entropy-based method, as described in Table 6.18.

Table 6.17: List of Markers Used for Gene-Gene Interaction Analysis in Cardiomyopathy

Gene	Markers	Code of the markers
ACE	(rs1799752) I\D	SNP1
MYH7	(rs397516208) G>A	SNP2
MYBPC3	(rs36212066) Δ25bp	SNP3
BAG 3	(rs2234962) T>C	SNP4

Table 6.18: Color Code Used for the Interpretation of the Results of Interaction Analysis.

Colour	Interpretations
Red	Highly Synergistic
Orange	Moderately Synergistic
Blue	Highly Redundant
Green	Moderately Redundant
Gold	Neither Synergistic/Redundant

6.5.1 Analysis of Interaction on the Study Population

Multifactor Dimensionality Reduction (MDR) analysis results evaluated all possible combinations of the studied polymorphisms in relation to the risk of developing cardiomyopathy disease (CM) among cases and controls from the Jammu & Kashmir population. A strong synergistic interaction between SNPs in SNPs that the combined effect of two genes (SNPS) on a phenotype is significantly greater than the sum of their individual effects. This suggests that the SNPs interact in a cooperative manner, amplifying their overall impact and resulting in a stronger association with the phenotype than would be expected if each acted independently. The hierarchical clustering dendrogram generated through MDR analysis (Figure 6.14) demonstrated two major clusters among the studied SNPS. The SNP2 (MYH7) and SNP4 (BAG3) are connected through a red branch, indicating the strongest synergistic interaction between two markers. The grouping pattern highlights that genetic variations in MYH7

and BAG3 might jointly modulate cardiac structural remodelling and apoptosis shown in Figure 6.14. In contrast, SNP 1 (ACE) and SNP3 (MYBPC3) are connected through blue branches this shows highly redundant effect that collectively could not influence vascular dynamics and sarcomere stability in disease susceptibility, but they have an independent effect. However, all the markers (SNPs) are connected through green branches, which shows the moderately redundant effect or less interaction of all the genes on the phenotype of Jammu populations.

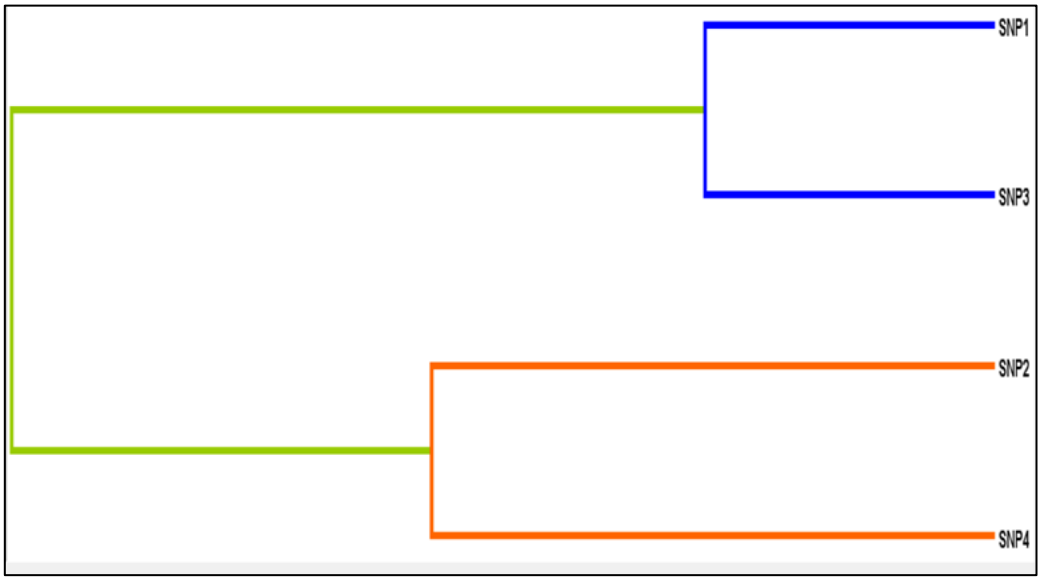


Figure 6.14: Representation of Dendrogram for the Interaction of selected genetic variants (ACE, MYH7, MYBPC3, and BAG3) in the population of Jammu Region.

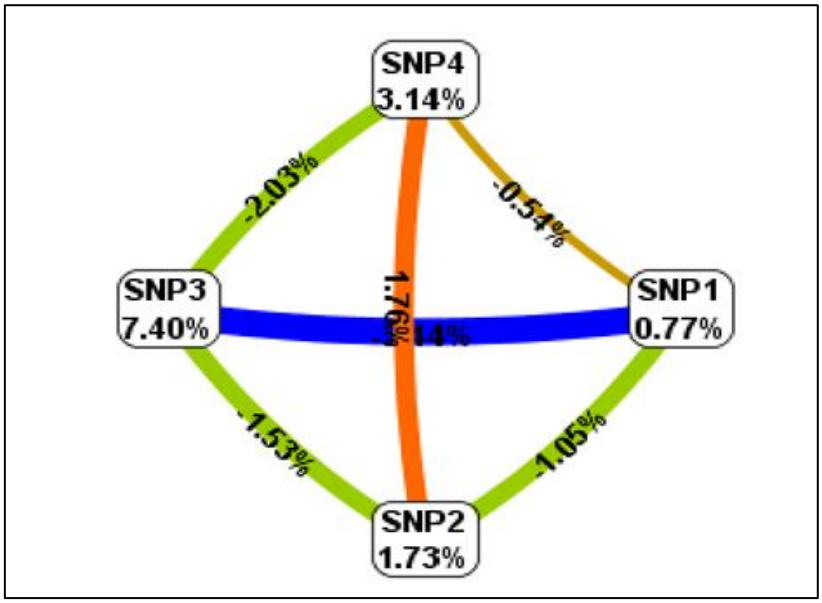


Figure 6.15: Representation of Best Fit Model of SNP–SNP Interaction Effect among *ACE, MYH7, MYBPC3*, and *BAG3* Genes Using MDR Analysis

The MDR circle graph (Figure 6.15) evaluated the strength of gene–gene interactions among *ACE* (SNP1), *MYH7* (SNP2), *MYBPC3* (SNP3), and *BAG3* (SNP4) based on entropy gain percentages. The strongest interaction was observed between *MYH7* (SNP2) and *BAG3* (SNP4), with an information gain of 17.04%, illustrated by a thick orange connecting line, indicating a robust synergistic effect. A moderate interaction was observed between *ACE* (SNP1) and *MYBPC3* (SNP3), with a 7.40% information gain, depicted by a thick blue line. Comparatively, other interactions between SNPs were weaker, with minor positive or negative entropy changes. These findings suggest that the combination of *MYH7* and *BAG3* polymorphisms may dominate cardiac disease pathogenesis, while *ACE* and *MYBPC3* jointly contribute through vascular and structural pathways. The MDR circle graph thus highlights the nonlinear synergistic effects of genetic variants that conventional single SNP analyses might overlook.

CHAPTER-7
DISCUSSION

7.1 Overview of Cardiomyopathy in the Context of Regional Epidemiology

This section introduces cardiomyopathy as a multifactorial disease and underscores the rationale for investigating its determinants in the demographically distinct Jammu region. Cardiomyopathy encompasses a diverse range of myocardial disorders that lead to cardiac dysfunction, failure of heart, arrhythmias, and, in some cases, cardiac death (Seferović et al., 2019). Historically regarded as primarily genetic in origin, emerging evidence now indicates that its development is altered by both genetic and environmental factors. In India, where cardiovascular disease has emerged as the major cause of mortality, the prevalence of cardiomyopathy is particularly concerning (McBride et al., 2015). However, it remains underexplored, especially within demographically and genetically distinct populations like those in the Jammu region of Jammu and Kashmir (Prabhakaran et al., 2018). This region, characterized by its ethnic diversity and distinct environmental conditions, offers a valuable opportunity to investigate how specific gene polymorphisms particularly those in sarcomeric, cytoskeletal, and mitochondrial genes interact with modifiable lifestyle factors such as diet, physical activity, smoking, alcohol consumption, and metabolic comorbidities including diabetes, hypertension, and obesity (Karasik et al., 2008). Gaining a deeper understanding of these interactions is crucial to deciphering the regional phenotypic variability of cardiomyopathy and creating tailored approaches for early detection and intervention (Burke et al., 2016). The present case-control study conducted in the Jammu region provides a comprehensive examination of the clinical, biochemical, lifestyle, and genetic risk factors associated with cardiomyopathy. Our findings contribute to and expand the existing literature on the multifactorial origins of cardiomyopathy. In this discussion, we compare our results with those of previous studies, emphasising both convergent and divergent findings to contextualise our research within the broader scientific landscape.

7.2 Distribution and Clinical Profile of Cardiomyopathy Subtypes

This section presents the distribution of cardiomyopathy subtypes observed in the study population and contextualises the findings by comparing them with national and international trends. The present study included a cohort of 250 clinically confirmed cardiomyopathy patients from the Jammu region. Among these, Dilated

Cardiomyopathy (DCM) emerged as the predominant subtype, representing 85.6% of all cases. This was followed by Hypertrophic Cardiomyopathy (HCM) in 11.2%, Arrhythmogenic Cardiomyopathy (ARCM) in 1.2%, and Restrictive Cardiomyopathy (RCM) in 0.8% of participants. The predominance of DCM in the present study is validating the findings reported by Kakarla et al. (2023), who also identified DCM as the most common cardiomyopathy subtype across diverse Indian populations, including both northern and southern regions (Kakarla et al., 2023). This reinforces the trend of a higher burden of DCM in Indian cohorts, including the Jammu population. In contrast, Neubauer et al. (2019) reported a different cardiomyopathy profile in Western populations, where HCM tends to be the most frequently diagnosed subtype, followed by a comparatively higher prevalence of RCM. This discrepancy highlights potential geographic, genetic, and environmental influences on cardiomyopathy subtype distribution, underscoring the importance of region-specific data in informing diagnostic and management strategies (Neubauer et al., 2019).

7.3 Symptomatology and Functional Limitations

Cardiomyopathy manifests with a broad spectrum of clinical symptoms that profoundly impact patients' quality of life and physical functioning. This section explores the symptom profile within the study cohort, with particular emphasis on the prevalence and functional implications of dyspnea and exertional intolerance. In the present study, 53.6% of participants reported experiencing shortness of breath, while 45.6% remained asymptomatic, and a small fraction (0.8%) reported only occasional dyspnea. Notably, exertional dyspnea was markedly more common, with 84.8% of individuals reporting difficulty in breathing while walking uphill underscoring a high burden of exercise intolerance in this population.

Chest pain or discomfort was another frequently reported symptom, affecting 59.2% of participants. In contrast, 25.6% reported no chest discomfort, and 15.2% experienced it only intermittently. These findings are in concordance with prior studies. For instance, Santos et al. (2016) documented a high prevalence of dyspnea among cardiomyopathy patients (Santos et al., 2016), while Neder et al. (2020) emphasized exertional dyspnea as a significant determinant of reduced functional capacity in this group (Neder et al., 2020). The current study's findings on chest pain parallel those of Paldino et al., (2022), who reported a wide variability in chest symptoms across

different cardiomyopathy phenotypes, reflecting the clinical heterogeneity of the disease (Paldino et al., 2022).

Collectively, these results not only corroborate previous literature but also reinforce the need for comprehensive symptom assessment in cardiomyopathy management, particularly in identifying functionally limiting symptoms such as dyspnea on exertion and chest discomfort, which have tangible implications for daily activity levels and patient care planning.

7.4 Demographic and Anthropometric Characteristics

This section explores the demographic and anthropometric characteristics of the study cohort, with a focus on age, sex distribution, and body mass index (BMI), while drawing comparisons with national and international findings. In the current study, 60.8% of participants diagnosed with cardiomyopathy were male, a pattern that reflects global epidemiological trends. This observation is consistent with the findings of Schneider et al. (2013), who reported a male predisposition to cardiomyopathy in diverse populations (Schneider et al., 2013). Various hypotheses have been proposed to explain this gender disparity, including the influence of androgenic hormones on gene expression, greater exposure to behavioural risk factors such as tobacco and alcohol, and the possible underdiagnosis in females due to atypical symptom presentation or healthcare access disparities.

The mean age of patients in this study was 57.7 years, which is slightly higher than the mean ages reported in similar cohorts from northern India, as noted by Meisters et al., (2022). This variation may be attributable to regional differences in lifestyle patterns, age at diagnosis, and access to early screening or preventive care. It may also suggest a delayed clinical presentation or diagnosis in the studied population.

In terms of anthropometry, the mean BMI in the cardiomyopathy group was 22.63 kg/m^2, marginally higher than in controls, though the difference did not reach statistical significance. However, a notable sex-based disparity was observed, with males constituting a larger proportion of the affected group. This supports previous findings from the Inter-heart study (Sagris et al., 2022), which identified dyslipidemia and other metabolic abnormalities as leading modifiable risk factors contributing to myocardial damage and the subsequent development of cardiomyopathy.

Overall, these demographic insights reinforce the multifactorial nature of cardiomyopathy risk and highlight the need for sex- and region-specific preventive strategies, including early screening for metabolic risk factors and tailored public health interventions.

7.5 Biochemical Risk Profile

Biochemical markers offer critical insights into the physiological and metabolic disturbances associated with chronic illnesses such as cardiomyopathy. In this study, the biochemical profile of individuals with cardiomyopathy revealed several notable deviations from healthy controls, underscoring the metabolic underpinnings of the disease. These findings are consistent with established literature on cardiometabolic dysfunction.

Notably, individuals in the cardiomyopathy group exhibited elevated levels of total cholesterol (236.42±44.28 mg/dL) and triglycerides, which are hallmark indicators of dyslipidemia and cardiovascular risk. These findings align with the work of Ma et al. (2020), who reported significantly higher lipid levels in patients with metabolic syndrome and type 2 diabetes, both of which are known contributors to structural and functional cardiac abnormalities (Ma et al., 2014). In addition, Kaur et al. (2021) demonstrated a clear correlation between hypertriglyceridemia and insulin resistance, further supporting the metabolic component of cardiomyopathy pathophysiology (Kaur et al., 2021). The pathophysiological significance of lipid abnormalities is also reinforced by a large-scale cohort study, which identified dyslipidemia as a major predictor of cardiovascular events, lending further credibility to the present findings (Chatterjee et al., 2019).

Moreover, the study found significantly lower HDL cholesterol levels (42.96 mg/dL) in the cardiomyopathy group compared to controls. Low HDL is indicative of impaired reverse cholesterol transport and reduced anti-inflammatory protection, both of which are important in the progression of atherosclerotic and cardiomyopathic changes. These findings are consistent with Wilkins et al. (2014), who described an inverse relationship between HDL levels and coronary heart disease risk. Similarly, Fotakis et al. (2019) emphasised the atheroprotective and anti-inflammatory functions of HDL, which are compromised in conditions marked by reduced HDL levels.

Supporting this perspective, Mahdy et al., (2012) proposed that even modest reductions in HDL can disproportionately elevate cardiovascular risk, particularly in genetically or metabolically vulnerable populations.

7.6 Electrolyte Homeostasis and Renal Function Indicators

Electrolyte balance and renal function are pivotal in maintaining cardiovascular stability, and their disruption often mirrors underlying cardiac pathologies. This section delves into the biochemical landscape associated with cardiomyopathy, with a focus on electrolyte imbalances and renal biomarkers that may reflect disease severity and progression. The biochemical alterations observed in the disease group—elevated sodium and potassium levels, reduced urea, and increased SGPT—indicate early signs of renal stress, altered protein metabolism, and hepatic involvement. The elevated sodium and potassium levels are consistent with findings by Chapman et al. (2024), who noted fluid imbalance and impaired renal clearance in cardiomyopathy patients. Interestingly, the lower urea levels, as discussed by Laville et al. (2023), may suggest reduced protein intake or diminished muscle mass, which is atypical in renal dysfunction.

The results of this study highlight the critical role of lifestyle factors in the onset and progression of cardiomyopathy. These factors, such as dietary habits, substance use, and metabolic health conditions, align with prior research that underscores their significance in cardiovascular diseases (CMS). Caffeine intake was identified as a strong risk factor for cardiomyopathy, showing a significant association ($p < 0.001$) with the condition and a moderate-to-strong effect size. This aligns with Grant et al., (2023), who found that excessive caffeine intake can elevate heart rate and blood pressure, which are known contributors to cardiac remodelling and dysfunction. Similarly, Zuchinali et al. (2016) linked chronic caffeine consumption to an increased risk of arrhythmias and other cardiac disturbances. Consequently, moderating caffeine intake may serve as an important preventive strategy for cardiomyopathy.

7.7 Lifestyle and Clinical Risk Factors Associated with Cardiomyopathy

Understanding modifiable lifestyle and clinical risk factors is key to addressing the rising burden of cardiomyopathy. This section highlights the influence of behaviours such as smoking and alcohol use, clinical conditions such as hypertension

and diabetes, dietary habits, and gender differences on disease risk and progression. Tobacco use and smoking emerged as the most strongly associated factors with cardiomyopathy in this study ($p < 0.001$), consistent with a range of epidemiological and mechanistic studies identifying these behaviours as major cardiovascular risk factors. Zhang et al. (2023) highlighted the role of smoking in promoting endothelial dysfunction, oxidative stress, and myocardial injury, thereby increasing the likelihood of developing cardiomyopathy. Alcohol consumption also showed a robust association ($p < 0.001$), aligning with Fernández et al. (2020), who demonstrated that chronic alcohol use can lead to alcohol-induced cardiomyopathy through myocardial toxicity and disruption of cardiac metabolism. Clinically, hypertension and diabetes were significantly linked to cardiomyopathy, corroborating findings from the study by Tran et al. (2019), which showed that high blood pressure and hyperglycaemia contribute to cardiac remodelling, hypertrophy, and fibrosis. Dietary factors, such as milk product intake ($p < 0.001$) and dietary patterns, were also significantly associated with disease status, supporting the work of Briggs et al. (2017), who found that diets high in saturated fats and animal products increase cardiovascular risk, while plant-based diets provide a protective effect. A modest but significant gender-based association ($p = 0.003$) aligns with findings from Divoky et al., (2018), which describe gender differences in cardiomyopathy patterns, with men being more prone to reduced ejection fraction and women to preserved ejection fraction.

7.8 Risk Assessment and Genetic Insights in the Pathogenesis of Cardiomyopathy

Cardiomyopathy is a complex, multifactorial disease influenced by various demographic, lifestyle, dietary, and biochemical factors. The analysis of associated comorbidities and clinical correlations provides critical insights into the predictors and risk factors for cardiomyopathy, contributing to a better understanding of its pathophysiology. The binary logistic regression analysis provides valuable insights into the multifactorial nature of cardiomyopathy, highlighting key demographic, lifestyle, dietary, and biochemical predictors of disease risk.

The male predominance observed in this study (OR = 5.63) is consistent with previous epidemiological findings, such as those by Zhao et al. (2022), which suggest higher susceptibility among men due to hormonal differences, increased left ventricular

mass, and possibly more harmful health behaviours. Similarly, the association between hypertension and elevated cardiomyopathy risk is strongly supported by research, including studies by Masenga et al. (2023), which link hypertension as a primary contributor to both hypertrophic and dilated cardiomyopathies.

One of the most striking results from this analysis is the exceptionally high odds ratio for caffeine consumption, both regular and occasional. While this finding is novel in its magnitude, earlier research by Kim et al. (2021) found only mild associations between excessive caffeine intake and arrhythmic events, without clear links to structural heart disease. This discrepancy warrants further investigation into regional dietary patterns, genetic factors, or potential confounding variables specific to this study's population.

The protective effect of dairy products (OR = 0.011) aligns with studies such as those by Aryanugraha et al. (2024), which suggest that dairy may benefit cardiometabolic health through blood pressure regulation and anti-inflammatory effects. The non-significant yet suggestive trends related to non-vegetarian diets and oily foods echo findings by Wang et al., (2017), indicating that the quality of fats and protein sources, rather than diet type, may influence cardiomyopathy risk.

Substance use, including alcohol and tobacco, though borderline in significance, remains a well-recognised factor in cardiomyopathy development. Alcohol-induced cardiomyopathy is well-documented, as shown by Urbano-Dikalov et al. (2019), while tobacco use exacerbates oxidative damage and endothelial dysfunction, both factors involved in the progression of myocardial degeneration.

Elevated triglyceride levels (OR = 209.54) emerged as a strong predictor of cardiomyopathy in this study, reinforcing the significant role of dyslipidaemia in cardiovascular pathology. A U-shaped relationship with potassium levels was also observed, consistent with Phillips et al. (2019), who highlighted the arrhythmogenic risks associated with both hypokalaemia (low potassium) and hyperkalaemia (high potassium). Furthermore, SGPT and urea were significant predictors, suggesting broader metabolic and organ-level dysfunction, which aligns with findings by Chan et al., (2016) regarding the prognostic importance of hepatic and renal biomarkers in cardiovascular outcomes.

The study also investigated the genotypic and allelic distribution of ACE I/D (rs1799752) and *MYH7* (rs397516208) polymorphisms in cardiomyopathy patients from the Jammu region, revealing significant associations between certain genetic variants and heightened disease susceptibility.

For the *ACE* polymorphism (*I/D*), the DD genotype and D allele were more prevalent in cardiomyopathy patients than in controls. Logistic regression showed a strong correlation between the DD genotype and increased disease risk. This finding corroborates earlier studies, such as those by Ribichini et al. (2004), which reported a link between the D allele and elevated ACE levels, a factor implicated in ventricular remodelling and hypertrophy. Similarly, Judge et al. (2023) found a higher prevalence of the DD genotype in idiopathic dilated cardiomyopathy, linked to faster disease progression. Our results align with those of Nemenoff et al. (2002) and Sayed-Walsh et al. (2022), who also observed a higher frequency of the D allele in cases of heart failure and hypertrophic cardiomyopathy (HCM).

Indian studies, such as Bai et al. (2012), also identified similar trends, with the DD genotype associated with increased myocardial fibrosis and left ventricular hypertrophy among HCM patients. A meta-analysis by Yang et al., (2013) further supports the global association of the D allele with cardiomyopathy risk, noting ethnic variations in allele frequencies. However, some studies, like those by Alves et al. (2020) and in a northern Chinese cohort, found no significant link between ACE polymorphisms and cardiomyopathy, suggesting that environmental or epigenetic factors may modulate the gene-disease relationship.

Regarding the *MYH7 G>A* polymorphism, the A allele and AA genotype were more frequent in cardiomyopathy cases, suggesting a potential association with increased disease susceptibility. Gao et al. (2024) highlighted the role of *MYH7* mutations in inherited cardiomyopathies, particularly those involving heavy chain of β-myosin, a key protein for cardiac contractility. In this study, the AA genotype was nearly three times more common in the cardiomyopathy group, suggesting a pathogenic role for this variant.

This finding supports studies by Richard et al., (2003), who identified *MYH7* mutations as a frequent cause of familial hypertrophic cardiomyopathy, and Marian et

al. (2021), who found a strong link between *MYH7* mutations and dilated cardiomyopathy, especially in early-onset cases. Karkkainen and Peuhkurinen (2007) proposed that *MYH7* polymorphisms may impair sarcomere function, contributing to systolic dysfunction. These results are further supported by Hershkovitz et al., (2019), who identified significant genotype-phenotype correlations in *MYH7* mutation carriers, including altered left ventricular geometry and reduced ejection fraction, and Leopold et al. (2020), who addressed the role of *MYH7* mutations in affecting severity of cardiomyopathy phenotypes.

However, not all studies show consistent findings. Kalayinia et al. (2018) identified asymptomatic carriers of *MYH7* variants, suggesting variable penetrance and the need for further family-based studies. Despite these inconsistencies, the current study supports the association between the A allele and cardiomyopathy risk, highlighting its elevated presence in affected individuals.

7.9 Predictive and Correlational Insights on Telomere Length in Cardiomyopathy

This section provides an integrated analysis of telomere length (TL) as both a predictive biomarker and a correlational indicator in relation to cardiomyopathy, examining its associations with demographic and biochemical variables.

A binary logistic regression analysis was conducted to evaluate the adjusted relationship between telomere length and cardiomyopathy, controlling for demographic and metabolic confounders. Telomere length emerged as a significant independent predictor, even after adjusting for age, gender, and BMI, indicating its robust potential in predicting disease risk. The findings of Fasching et al. (2018) and Engel et al. (2021) are consistent with our results that underscore telomere shortening as a marker of biological ageing and cardiovascular vulnerability.

In terms of demographic associations, an evident decline in telomere length corresponding to age was detected in cardiomyopathy patients. This trend mirrors the findings of Muthamil et al. (2024) and Fyhrquist et al. (2013), who documented accelerated telomere attrition with ageing and cardiovascular burden. Gender-based differences were also evident, with females exhibiting longer telomeres, supporting the estrogen-related protective hypothesis previously discussed in studies such as Assalve

et al., (2025) (Demanelis et al., 2021; Zafirovic et al., 2022). Additionally, BMI showed a negative correlation with TL, consistent with findings of Taheri et al., (2022) that link obesity to chronic inflammation and increased oxidative stress (Weischer et al., 2012)

On the biochemical front, significant negative correlations were found between TL and triglycerides, SGPT, and total cholesterol, highlighting the influence of metabolic stress on telomere erosion. These results align with studies by Liu et al., (2023), Kim et al. (2018), and Shams et al. (2011), which link dyslipidemia and liver dysfunction to telomere attrition via oxidative and inflammatory pathways.

Although the positive correlation between HDL and TL in this study was not statistically significant, the trend supports the antioxidant and anti-inflammatory role of HDL suggested in earlier literature by Zerach et al. (2020). A moderate inverse correlation between potassium and cholesterol was also identified, echoing findings by Zafirovic et al. (2022), who noted such patterns in familial hypercholesterolemia.

While sodium and urea levels did not demonstrate significant associations with TL, their weak correlations suggest a limited direct role in telomere dynamics. Nevertheless, the overall pattern of findings reinforces the interpretation that telomere length reflects cumulative physiological and metabolic stress over time. Finally, these results are supported by meta-analytical evidence such as that from Weischer et al. (2012), which showed that each standard deviation decrease in TL corresponds to a 21% increased risk of coronary artery disease.

7.10 Gene-Gene Interactions in Disease Susceptibility

The genetic underpinnings of disease susceptibility require considering the complex interactions between multiple genes, as these interactions often play a critical role in determining disease risk. Additionally, gene-gene interaction analysis through MDR revealed synergistic interactions, especially between *MYH7* and *BAG3,* in modulating disease susceptibility. This is supported by prior studies such as Kirk et al. (2021), which identified *MYH7* and *BAG3* mutations as critical contributors to structural remodelling and apoptosis in cardiac tissues. These interactions possibly compound telomere dysfunction by exacerbating myocardial stress and cellular senescence.

Herewith, the present case-control study conducted in the Jammu region provides a detailed assessment of the multifactorial aetiology of cardiomyopathy, highlighting the interplay of genetic, clinical, biochemical, and lifestyle factors. Dilated cardiomyopathy (DCM) emerged as the predominant form, consistent with national trends. Significant genetic associations were identified with polymorphisms in the *ACE (I/D), MYH7 (G>A)*, and *MYBPC3* genes. The *ACE* D allele and DD genotype were linked to increased susceptibility due to their role in ventricular remodelling; the *MYH7* A allele and AA genotype suggested impaired myocardial contractility; and *MYBPC3 25bp* deletion and *BAG3* variants were implicated in structural cardiac abnormalities. Shortened telomeres were identified as an independent predictor of the disease, reflecting cellular ageing and physiological stress. Lifestyle risk factors such as tobacco use, alcohol consumption, and caffeine intake showed strong associations with cardiomyopathy. Biochemical abnormalities included dyslipidaemia (elevated cholesterol and triglycerides, reduced HDL), electrolyte imbalances, and indicators of renal and hepatic dysfunction. These findings emphasise the need for integrated, region-specific strategies that consider both genetic predisposition and modifiable risk factors in the management of cardiomyopathy. Consequently, this discussion highlights the need for more research as well as the potential benefits of the study's findings for cardiomyopathy research and therapy.

CHAPTER-8
SUMMARY AND CONCLUSIONS

8.1 Summary

This study provides an in-depth analysis of the multifactorial aetiology of cardiomyopathy in the Jammu region, integrating genetic, clinical, biochemical, and lifestyle variables. The findings underscore the complex interplay between modifiable risk factors, such as metabolic parameters and lifestyle habits, and non-modifiable contributors, including genetic polymorphisms. The study enhances our understanding of the disease's underlying mechanisms by capturing the heterogeneity of cardiomyopathy phenotypes and identifying independent risk factors. Among the 250 clinically confirmed cases, **Dilated Cardiomyopathy (DCM)** emerged as the most prevalent subtype (85%), followed by **Hypertrophic Cardiomyopathy (HCM)** (11%). Other subtypes, such as Restrictive Cardiomyopathy (RCM), Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC), Peripartum Cardiomyopathy (PCM), and Ventricular Cardiomyopathy (VCM), collectively constituted only 4%. The predominance of DCM aligns with both national and global trends and may reflect underlying genetic susceptibility, environmental exposures, and diagnostic limitations in the region. Clinically, **dyspnea** and **chest pain** were common across subtypes. DCM cases presented with congestive symptoms like fatigue and edema, whereas HCM cases often exhibited exertional chest pain and palpitations. These symptom variations underscore the importance of phenotype-specific diagnostic and treatment approaches.

Demographic Factors Analysis revealed a significant male predominance, suggesting gender-based biological or lifestyle differences in disease susceptibility. While differences in BMI and age were not statistically significant, patients displayed higher BMI trends, indicating a potential link between adiposity and cardiomyopathy pathogenesis.

Biochemically, elevated levels of total cholesterol, triglycerides, and SGPT, along with reduced HDL and electrolyte imbalances, indicate systemic metabolic dysregulation. These results support a cardiometabolic model of illness and suggest hepatic involvement, which may be connected to non-alcoholic fatty liver disease (NAFLD). Low serum urea levels may reflect impaired protein metabolism or early cardiorenal dysfunction.

Lifestyle Factors, such as high caffeine intake, tobacco consumption, alcohol consumption, and smoking, were all significantly associated with increased cardiomyopathy risk. Comorbidities such as **hypertension** and **type 2 diabetes** further exacerbated disease severity, highlighting the need for integrated management strategies.

Dietary Habits—specifically reduced milk intake and a non-vegetarian diet—were also associated with disease risk, potentially through effects on lipid metabolism and systemic inflammation.

Genetic Analysis revealed strong correlations between cardiomyopathy and specific polymorphisms: ACE I/D polymorphism. A Higher frequency of the DD genotype was observed among patients, indicating a strong association with disease in all inheritance models. This may reflect ACE's role in vascular and myocardial remodelling. **MYH7 gene**: The A allele showed a dose-dependent relationship with cardiomyopathy, with AA genotype carriers exhibiting significantly higher disease risk. **MYBPC3 gene**: The DD genotype 25bp deletion shows genetic association with the disease in our population. **BAG3 gene**: The CC genotype was significantly associated with disease, supporting BAG3's role in protein quality control and cardiomyocyte survival.

Telomere Length (TL) was significantly shorter in patients, particularly among older males with higher BMI. TL was negatively correlated with triglycerides, SGPT, and BMI, linking it to metabolic stress and inflammation. Logistic regression confirmed TL as an independent predictor, reinforcing its value as a biomarker for biological ageing and disease susceptibility.

Multifactor Dimensionality Reduction (MDR) Analysis Revealed

Synergistic Interactions between ***MYH7*** **and** ***BAG3,*** amplifying cardiomyopathy risk.

Redundant Effects between ***ACE*** **and** ***MYBPC3,*** where each gene acted independently. These findings highlight the role of gene-gene interactions and suggest that a complex genetic network shapes cardiomyopathy risk.

8.2 Conclusion

This study presents critical insights into the genetic, metabolic, and lifestyle-related factors contributing to cardiomyopathy development in the Jammu region. It confirms the multifactorial nature of the disease, characterised by significant variability in clinical presentation, biochemical profiles, and genetic backgrounds.

The independent association of telomere length with cardiomyopathy, alongside established genetic markers such as *ACE, MYH7, MYBPC3*, and *BAG3*, positions TL as a promising early detection and risk stratification biomarker. Similarly, the strong correlations of modifiable lifestyle and dietary factors with disease risk underline the importance of targeted preventive strategies.

Future research should focus mostly on longitudinal studies to identify causal relationships and explore the utility of these markers in predicting disease progression and response to interventions. The integration of genetic and metabolic screening in routine clinical practice could facilitate early diagnosis, individualised care, and more effective public health planning.

8.3 Future Directions and Recommendations

i. Expanding the sample size to include diverse, multi-ethnic populations will help validate the regional specificity of the observed genetic associations and ensure broader applicability.

ii. Incorporating genetic screening into routine clinical practice, especially for individuals with a family history of cardiomyopathy, could help identify high-risk patients early on.

iii. Further functional investigations are needed to illustrate the specific mechanistic pathways with identified polymorphisms (*ACE, MYH7, MYBPC3, BAG3*) that influence cardiomyopathy development, particularly focusing on the molecular and cellular effects in cardiac tissues.

iv. Given the strong associations between lifestyle factors (e.g., smoking, alcohol consumption, and diet) and cardiomyopathy, public health initiatives should emphasise the importance of healthy lifestyle choices in mitigating disease risk.

v. Exploration of potential therapeutic strategies targeting high-risk genotypes or metabolic dysregulations could be valuable.

vi. Routine biochemical monitoring, including lipid profiles, liver function tests (e.g., SGPT), and telomere length, should be incorporated into clinical assessments to provide early indicators of disease onset and progression.

vii. Collaborative efforts between researchers, clinicians, and policymakers are essential to translate research findings into effective public health strategies.

8.4 Limitations

Although the study includes many participants, a larger sample size is required for more robust statistical power and to account for potential ethnic variations in genetic associations. The study's cross-sectional nature limits the ability to establish causality between genetic factors, lifestyle factors, and cardiomyopathy. The exclusion of certain genotypes, such as heterozygotes for *MYBPC3*, may be due to technical limitations in detection or population-specific variations. Although the study provides a keen understanding of the prevalence and risk factors related to cardiomyopathy, it does not address the impact of therapeutic interventions, which would be important in future research to evaluate potential treatment options.

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APPENDICES

GOVERNMENT MEDICAL COLLEGE, JAMMU
INSTITUTIONAL ETHICS COMMITTEE

(IEC,GMCJ Registration No.: ECR/454/Inst/JK/2013/RR-20, Dated: 26-05-2020)

CERTIFICATE

Shikha Bharti
PHD Scholar
LPU Jalandhar.

Ref: Project No. **A-20**

No: - IEC/GMCJ/2022/1143 **Dated :-03.11.2022**

Dear Investigator

Institutional Ethics Sub-Committee reviewed and discussed your application for expedited review for provisional approval of the research study titled ***" STUDY OF GENE POLYMORPHISM AND NON-GENETIC FACTORS ASSOCIATED WITH CARDIOMYOPATHY PHENOTYPES IN THE JAMMU REGION OF JAMMU AND KASHMIR "*** on dated :**15.10.2022**

The following documents were reviewed and approved (Applicable/Non Applicable).

1. Project Submission form.
2. Study Protocol (including protocol amendments), (Yes/No).
3. Patient information sheet and informed consent form (including updates if any) in English and/Vernacular language. (Applicable/ Non Applicable)
4. Investigator's Brochure, dated _____, version no. _____ (Applicable/Non Applicable)
5. Case Record Form (Applicable/Non Applicable)
6. Current CVs of Principal Investigator, Co-Investigators. **(PHD Scholar)**
7. Package inserts (Applicable/ Non Applicable)
8. Insurance Policy/ compensation for participation and for serious adverse events occurring during the study participation. (Applicable/Non Applicable).
9. Investigators' Agreement with the sponsor. (Applicable/ Non Applicable).
10. Investigator's undertaking. (Applicable/ Non Applicable)
11. DCGI/ Regulatory Authority approval (Applicable/ Non Applicable)
12. Clinical Trial Agreement (CTA)/ Memorandum of Understanding (MoU) / Material Transfer Agreement (MTA) if applicable (Applicable/ Non Applicable)

DECISIONS OF IEC IS TO:

- **Approved**
- Revision with minor modifications/amendments
- Revision with major modifications for resubmission
- Resubmit
- Not approved
- Deferred
- Query- Further clarification/modification required

Prof (Dr.) Sanjay Kumar Bhasin
Member Secretary
Institutional Ethics Committee
Govt. Medical College Jammu

Member Secretary
Institutional Ethics Committee
Govt. Medical College
Jammu

To whosoever it may concern

It is confirmed that **Shikha Bharti**, bearing registration number **42100155**, is registered as a full time PhD scholar in the Department of Zoology, Lovely Professional University, Punjab, India. For her PhD, she is working on **"STUDIES ON GENE POLYMORPHISM AND NON-GENETIC FACTORS ASSOCIATED WITH CARDIOMYOPATHY PHENOTYPES IN THE JAMMU REGION OF JAMMU AND KASHMIR"**. As this is a clinical study, and a part of this study involves collection of samples from different patients enrolled in various hospitals of Jammu region, and for that she needs to have ethical clearance from the parent institute as well as the concerned hospitals. This is to inform you that our Institutional Ethics Committee is in process of registration with the Department of Health Research (DHR), New Delhi and till the time we get the final registration, this letter should be regarded as "**NO OBJECTION**" certificate from the University for the concerned student to conduct this study. We hope for your kind consideration.

NRSharma 21/01/2023

Dr. Neeta Raj Sharma ,

Professor & Dean ,

School of Bioengineering & Biosciences.

Jalandhar-Delhi G.T.Road, Phagwara, Punjab (India) - 144411
Ph : +91-1824-444050 Fax : +91-1824-506111 E-mail : neeta.raj@lpu.co.in website : www.lpu.in

DEPARTMENT OF ZOOLOGY LOVELY PROFESSIONAL UNIVERSITY & INSTITUTE OF HUMAN GENETICS UNIVERSITY OF JAMMU, JAMMU

CONSENT FORM

I have been explained the possible risk and benefits and have understood purpose for which my blood sample is being sought by the Institute Of Human Genetics, University Of Jammu. I am free from any pressure what so ever and hereby give my own consent to:

I. The withdrawal of sample of about 2ml/3ml blood by venipuncture.
II. To all type of analysis of my blood for non-profit research purposes for acquisition of knowledge, for the benefit to the mankind by Institute Of Human Genetics, Jammu or their direct collaborators.

I will have the right to know the analyzed result of my blood sample and I am not giving my consent for disclosure of any personal information but the Institute Of Human Genetics, University of Jammu may publish this data in National /International journal in the coded form.

Signature/Thumb Impression

Name:-

Age:-

Gender:-

Address:-

Name of the Collector:-

Date:-

Deputy Coordinator,

Institute Of Human Genetics,

University Of Jammu.

Department of Zoology Lovely Professional University
& Institute of Human Genetics
University of Jammu, Jammu J&K 180006
ISO 9001-2000 Certified

Research questionnaire:

A. Personal Information

Name:_____, Gender_____, Address (DISSTT only)_____, Age_____Weight_____, Height_____, Blood Group_____, Occupation_____

Religion_____ Marital status_____

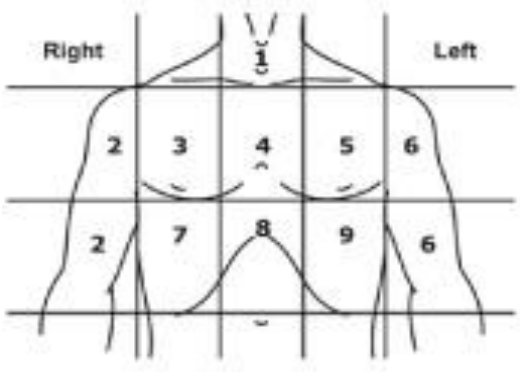
1. Do you have ever heart attack? **Yes/ No**
2. Have you ever had any pain or discomfort in your chest? **Yes/ No/ occasionally/No idea**
3. Do you get it when you walk uphill?**Yes/ No/ Never walk uphill/ No idea**
4. Do you have pain/ discomfort in chest when you walk at an ordinary pace?**Yes/ No/ No idea**
5. What do you do if you get the pain while you are walking?

i) Stop walking ii) slow down iii) carry on

6. How soon the pain will occur?

i) 10 Minutes ii) less than 10 mins iii) more than 10 min

7. In which area of your chest you feel the pain?



Please tick the number 1,2,3,4, 5,6,7,8,9

8. Have you have ever had a severe pain across the front of your chest lasting for half an hour or more? **Yes/ No/ may be/ No idea**
9. Have you ever shortness of breath other than when you have cold? **Yes/ No/ May be/ No idea**

10. Any members of your family have these symptoms? Yes/ No/ may be/ no idea
11. If any members of your family have these symptoms what relation do you have with him/her? _____.

B. Technique on which disease diagnosis:

ECG, ECOCARDIOGRAPHY, BLOOD TEST, CARDIAC MRI, CARDIAC CT SCAN CHEST XRAYS, NONE

C. RISK FACTOR

1. Sleep time ___:___ PM/AM and Wake Up time: ___:___AM
2. Stress: No Stress/ Educational stress/ Emotional Stress/ Occupational Stress/ physical/ Health related.
3. What kind of food do you take? Veg./ Non-veg.
4. Do you consume oily food? Yes/ No/ Occasionally
5. Do you consume Junk food? Yes/ No/ Occasionally
6. Do you consume Milk/ dairy products? Yes/ No/ Occasionally
7. Caffeine intake? Yes/ No/ Occasionally
8. Tea with Salt? Yes/ No/ Occasionally
9. Tea with Salt? Yes/ No/ Occasionally
10. Water intake per day? _____ltr
11. Physical activity? Gym/ Sports/ Jogging/ yoga/ House Hold work/ other.
12. **Smoking**? Yes/ No/ Occasionally
13. **Alcohol**? Yes/ No/ Occasionally
14. **Tobacco:** (a) Inhale:Yes/No/ Occasionally (b) Chewing Tobacco: Yes/No/ Occasionally.
15. Fasting? Yes/ No/ Occasionally
16. Any disease: Hypertension, diabetes, cardiovascular disease, cholesterol, obesity, kidney stone, headache, PCOS. Other_____.

Signature of the subject

Name of the collector Supervisor signature

Pedigree:

Reference: Centers for Disease Control and Prevention (Government Agency, Rahman et al., 2013

Department of Zoology Lovely Professional University
& Institute of Human Genetics
University of Jammu, Jammu J&K 180006
ISO 9001-2000 Certified

Research questionnaire: control

Personal Information

Name: _____,Gender_____,Address (DISSTT only)_____, Age_____ Weight_____, Height_____, Blood Group_____, Occupation_____ Religion_____ Marital status_____

1. Do you have ever heart attack? **Yes/ No**
2. Sleep time ___:___ PM/AM and Wake Up time: ___:___AM
3. Stress: No Stress/ Educational stress/ Emotional Stress/ Occupational Stress/ physical/ Health related.
4. What kind of food do you take? Veg./ Non-veg.
5. Do you consume oily food? Yes/ No/ Occasionally
6. Do you consume Junk food? Yes/ No/ Occasionally
7. Do you consume Milk/ dairy products? Yes/ No/ Occasionally
8. Caffeine intake with salt or with sugar? Yes/ No/ Occasionally
9. Tea with Salt? Yes/ No/ Occasionally (one/ two/ three cups)
10. Tea with sugar? Yes/ No/ Occasionally
11. Water intake per day? _____ltr
12. Physical activity? Gym/ Sports/ Jogging/ yoga/ House Hold work/ other/ walk/ No.
13. **Smoking**? Yes/ No/ Occasionally
14. **Alcohol**? Yes/ No/ Occasionally

    Regular / twice in a week/ thrice in a week
15. **Tobacco:** (a) Inhale: Yes/No/ Occasionally b) Chewing Tobacco: Yes/No/Occasionally.
16. Fasting? Yes/ No/ Occasionally

Signature of the subject Date:

CERTIFICATE OF CONFERENCES & WORKSHOPS

LIST OF CONFERENCES

1. **Poster Presentation: Shikha Bharti**, Najitha Banu, Parvinder Kumar. Genetic Association of the ACE Gene Polymorphism with Cardiomyopathy in Jammu Region. International Conference on Emerging Trends in Biosciences and Chemical Technology (ETBCT) 2025.
2. **Oral Presentation**: **Shikha Bharti**, Najitha Banu, Parvinder Kumar. MYH7 gene Polymorphism and susceptibility to cardiomyopathy: A case control study from Jammu Region. National conference on Emerging innovations in Biochemistry and Biotechnology for Holistic Development of Agriculture 2025.
3. **Participation: Shikha Bharti**, Najitha Banu, Parvinder Kumar. International Conference on Sustainability: Life on Earth 2021(ICS-LOE 2021).

LIST OF WORKSHOPS

1. **"Vritika Research Internship" SERB Sponsored**

   Date: 2 May,2022- 24 June,2022 **Location:** Punjab University, Chandigarh, India.

   Organizer: Department of Biophysics, Punjab University.

2. **7-Day workshop on "Atomic Absorption Spectroscopy (AAS) & Polymerase Chain Reaction (PCR)" DST Funded under STUTI**

   Date: 14 Nov,2022- 20 Nov,2022 **Location:** University of Jammu, J&K, India.

   Organizer: Department of Zoology, University of Jammu with IIT, Gandhi Nagar

3. **"Hands-on training workshop in Automated DNA Sequencing" JKGENOMICS**

   Date: 18 March 2023 **Location:** SMVDU, J&K, India.

   Organizer: Shri Mata Vaishno Devi University, Katra, J&K.

4. "**Hands-on Training in Advanced Bioinformatics Techniques in Molecular Biology" sponsored by HEC, J&K**

   Date: 7 March, 2024 – 12 March, 2024 **Location:** University of Jammu, J&K, India.

   Organizer: Department of Zoology, University of Jammu in Collaboration with Malaviya Mission Teacher Training centre (MMTTC), University of Jammu

5. **Three-day National Training program on "Bioinformatics: Basics, Methodology& Applications" organised by National Academy of Science India (NASI) Jammu Chapter**

   Date: 3 March,2024 – 5 March,2024 **Location:** University of Jammu, J&K, India.

   Organizer: Department of Botany, University of Jammu and National Academy of Sciences (NASI).

Annual Convention

Society for Plant Biochemistry and Biotechnology (SPBB)

Sher-e-Kashmir University of Agricultural Sciences & Technology- Jammu, Union Territory of Jammu & Kashmir, India

&

Division of Biochemistry, ICAR-IARI, New Delhi

Certificate of Participation

This is to certify that ..... SHIKHA BHARTI .....

has participated / presented ~~LEAD PAPER~~ / BEST ORAL / ~~POSTER~~ in Technical Session ... MOLECULAR ...

... APPROACHES TO ANIMAL HEALTH ..... Annual Convention of the Society for Plant Biochemistry and Biotechnology SPBB and National Conference on **"Emerging Innovations in Biochemistry and Biotechnology for Holistic Development of Agriculture"** held at Sher-e-Kashmir University of Agricultural Sciences & Technology- Jammu, Union Territory of Jammu & Kashmir, India during 06th-07th March, 2025.

(G.K. Rai)
Organizing Secretary
SKUAST-Jammu

(Aruna Tyagi)
Secretary, SPBB

(Ranjeet R. Kumar)
Organizing Secretary
ICAR-IARI

Department of BioTechnology, Government of India

सत्यमेव जयते

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विज्ञान एवं प्रौद्योगिकी मंत्रालय
MINISTRY OF SCIENCE AND TECHNOLOGY

International Conference On Emerging Trends In Biosciences And Chemical Technology-2025

February 14- 15, 2025

Organized by

School of Biotechnology, Shri Mata Vaishno Devi University, Katra

Certificate of Participation

This is to certify that **Ms. Shikha Bharti** has presented poster entitled **Genetic Association of the ACE Gene Polymorphism with Cardiomyopathy in Jammu Region** at International Conference on Emerging Trends in Biosciences and Chemical Technology (ETBCT-2025) held at Shri Mata Vaishno Devi University, Katra, J&K-India.

Dr. Indu Bhushan	Dr. Shafaq Rasool	Dr. Pravez Slathia	Prof. Ratna Chandra
Convener	Co-Convener	Co-Convener	Organizing Secretary

LIST OF PUBLICATIONS

Cardiology

Cardiovascular Biomarkers: Research Article

Cardiology
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Genetic Association of the Ins/Del Variant of *ACE* and Risk of Cardiomyopathy: A Case-Control Study and Updated Meta-Analysis

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Keywords
Cardiomyopathy · Dilated cardiomyopathy · Hypertrophic cardiomyopathy · Angiotensin-converting enzyme

Abstract

Introduction: Cardiomyopathy is a complex condition influenced by multiple genes and environmental factors. It has been suspected that cardiomyopathy is affected by the *ACE* gene's I/D polymorphism. Our study aimed to evaluate the association between this polymorphism and cardiomyopathy risk in the Jammu population of North India, alongside a meta-analysis to determine the specific risks associated with different types of cardiomyopathy. ***Method:*** In the case-control study, we opted for a convenient sampling technique to gather patients from hospitals. Meanwhile, for the meta-analysis registered under PROSPERO with CRD42024519763, and in line with PRISMA guidelines, we accessed online databases and applied predefined inclusion criteria. Data extraction and quality assessment were performed using the Newcastle-Ottawa scale. Statistical analysis included genotypic frequencies, Hardy-Weinberg equilibrium testing, logistic regression models, and assessments for heterogeneity and publication bias. ***Result:*** The case-control study revealed a significant association between the *ACE* I/D risk variant and cardiomyopathy risk in the Jammu population (odds ratio [OR]: 1.30, confidence interval [CI; 1.04–1.63], p value = 0.021). Furthermore, a total of 34 studies were fund-eligible for the meta-analysis and demonstrated a significant association between the risk variant and both dilated (OR: 1.25, CI [1.03–1.50], p value = 0.022) and hypertrophic (OR: 1.31, CI [1.0876–1.5776], p value = 0.004446) cardiomyopathy. ***Conclusion:*** Our study found a significant association between the I/D polymorphism and cardiomyopathy risk in the Jammu population. Further, the meta-analysis strengthens the findings by consistently linking the *ACE* I/D polymorphism to both dilated and hypertrophic cardiomyopathy. These results

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Review Article

Understanding the Etiopathogenesis of Cardiomyopathies: New Insights

Indian Journal of Clinical Cardiology
1–18

DOI: 10.1177/26324636241288394
journals.sagepub.com/home/occ
Sage

Shikha Bharti[1,2], Dharminder Kumar[3], Amrit Sudershan[2,4], Kanak Mahajan[2], Srishty Sudershan[5], Ashiq Hussain Mir [1], Najitha Banu[1] and Parvinder Kumar[2,5]

Abstract

Background: Cardiomyopathy, a rare heart disease, is characterized by abnormalities in cardiac wall thickness and chamber size, leading to impaired contraction, relaxation, conduction, rhythm, and reduced pumping ability.
Aim: This review aims to provide a comprehensive understanding of cardiomyopathy by examining its various aspects
Method: A literature survey was conducted using online databases such as PubMed, Google Scholar, and Web of Science, covering publications from January 1995 to July 2023.
Result: Genetic mutations in key muscle contraction genes (MYH7, MYL2, MYL3, MYBPC3, TNNT2, TPM1, TNNI3, ACTC) contribute to cardiomyopathy. Additionally, epigenetic markers in genes like FKBP5, TBX5, HAND1, POLA2, PLAAT3, and CCDC88B, along with environmental factors such as alcohol addiction, smoking, and stress, significantly influence disease risk. Genetic testing, including whole exome/genome sequencing, has revolutionized diagnosis, enabling early detection and intervention. Familial genetic testing facilitates personalized management.
Conclusion: Cardiomyopathy is a complex disease with genetic and environmental influences. Various techniques, including genetic testing, aid in its identification and management. Furthermore, machine learning (ML) techniques have emerged as valuable tools in understanding and predicting cardiomyopathy outcomes.

Keywords

MYH7, HCM, DCM, types of cardiomyopathies, risk factors

Received 11 November 2023; **accepted** 28 August 2024

Introduction

It is well recognized that the heart is the first organ to form in an embryo, and the development of the heart has a significant impact on the development of succeeding organs. However, heart disease poses a significant threat as a leading global cause of morbidity and mortality. This broad category includes conditions like congenital heart defects, valvular diseases, arrhythmias, heart failure, coronary artery disease, and cardiomyopathy. Cardiomyopathies, in particular, are complex disorders marked by altered heart chamber size, abnormal cardiac wall thickness, disturbed conduction, impaired relaxation and contraction, dysregulated rhythm, and reduced pumping ability.[1] The term "cardiomyopathy" was first introduced in 1957 by Wallace Brigden at the National Heart Hospital in London to describe a group of basic myocardial diseases. Subsequently, the WHO used the term to refer to heart diseases with no known cause. In 2006, the American Heart Association developed a modern classification system based on recent advancements, categorizing cardiomyopathies into hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), Arrhythmogenic Right

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OPEN ACCESS

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

Shikha Bharti[1,3], Pranay Kumar[4], Dharminder Kumar[5], Surbhi Pathania[3], Parvinder Kumar[2, *3], Najitha Banu[*1]

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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

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ORIGINAL ARTICLE

Impact of MYH7 gene polymorphism and lifestyle factors on cardiomyopathy susceptibility in Jammu: a case–control analysis

Shikha Bharti[1,5] · Shashank Garg[2] · Rakesh Kumar Panjaliya[4] · Dharminder Kumar[3] · Mohd. Younis[4] · Najitha Banu[1] · Parvinder Kumar[4,5]

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Abstract

Cardiomyopathy is a major risk factor for cardiac dysfunction, which is caused by a complicated interaction between environmental and hereditary variables. Changes in the MYH7 gene help to understand how heart muscle cells work and how these changes can lead to heart problems. This study examines the relationship between cardiac dysfunction in the Jammu population and the *MYH7* rs397516208 (G > A) variant. The study comprised 500 healthy controls and 250 patients with cardiomyopathy. PCR–RFLP was used to genotype the *MYH7* variant. Detailed statistical analyses revealed that patients had a considerably higher prevalence of the MYH7 (rs397516208) AA genotype than controls ($p < 0.001$), indicating a strong genetic link with heart dysfunction. Cholesterol is a significant contributor to the progression of heart disease, and a statistically significant association ($p < 0.05$) was found between the disease group and cholesterol levels. Also, alcohol consumption increases the risk of cardiomyopathy by twofold in people with AA variants (OR = 2.4 [CI = 1.075−22.91] $p < 0.04$). In conclusion, the *MYH7* (rs397516208) variant is genetically linked to cardiac dysfunction and may contribute to the pathophysiology of heart failure and related cardiac disorders.

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Significance Statement: This is the first report on the investigation on the molecular genetic analysis of the candidate genes associated with cardiomyopathies attendant in the population in the population of Kashmir region. Identification of the mutations and gene interactions that contribute to disease progression has not only enhanced the scientific understanding but also has clinical utility in guiding patient management and providing valuable insights for affected families.

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