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Hypertrophic cardiomyopathy in *MYBPC3* carriers in aging

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Abstract

Hypertrophic cardiomyopathy (HCM) is characterized by abnormal thickening of the myocardium, leading to arrhythmias, heart failure, and elevated risk of sudden cardiac death, particularly among the young. This inherited disease is predominantly caused by mutations in sarcomeric genes, among which those in the cardiac myosin binding protein-C3 (*MYBPC3*) gene are major contributors. HCM associated with *MYBPC3* mutations usually presents in the elderly and ranges from asymptomatic to symptomatic forms, affecting numerous cardiac functions and presenting significant health risks with a spectrum of clinical manifestations. Regulation of *MYBPC3* expression involves various transcriptional and translational mechanisms, yet the destiny of mutant *MYBPC3* mRNA and protein in late-onset HCM remains unclear. Pathogenesis related to *MYBPC3* mutations includes nonsense-mediated decay, alternative splicing, and ubiquitin-proteasome system events, leading to allelic imbalance and haploinsufficiency. Aging further exacerbates the severity of HCM in carriers of *MYBPC3* mutations. Advancements in high-throughput omics techniques have identified crucial molecular events and regulatory disruptions in cardiomyocytes expressing *MYBPC3* variants. This review assesses the pathogenic mechanisms that promote late-onset HCM through the lens of transcriptional, post-transcriptional, and post-translational modulation of *MYBPC3*, underscoring its significance in HCM across carriers. The review also evaluates the influence of aging on these processes and *MYBPC3* levels during HCM pathogenesis in the elderly. While pinpointing targets for novel medical interventions to conserve cardiac function remains challenging, the emergence of personalized omics offers promising avenues for future HCM treatments, particularly for late-onset cases.



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Keywords: Age-related HCM, *MYBPC3*, alternative splicing, nonsense-mediated decay, ubiquitin-proteasome system, chaperone-mediated autophagy

INTRODUCTION

Hypertrophic Cardiomyopathy (HCM) impacts as many as 1 in every 200 people^[1], affecting approximately 36 million people worldwide^[2,3]. HCM is characterized by thickening of the heart muscle, with or without left ventricular outflow obstruction, and is associated with diastolic dysfunction^[4,5]. Sudden cardiac death (SCD) is strongly linked to HCM, particularly in young adults and highly trained elite athletes^[6]. HCM is a primary genetic cause of pathological left ventricular hypertrophy and heart failure, a significant indicator of cardiac-related morbidity and mortality^[7-9]. Typically, treatments for HCM are pharmacologic drug administration to treat symptoms and, in some cases, myectomy to prevent progression to heart failure (HF)^[10,11]. Treating the precise fundamental defects of sarcomere function is critical to further progress in preventing HCM complications. HCM is caused predominantly by inherited mutations in the sarcomeric proteins, and it is the resultant contractile abnormalities that impair cardiac function^[12]. Over 50%-60% of all inherited HCMs^[13,14] are caused by mutations in the *MYBPC3* gene that encodes the cardiac isoform of the sarcomeric protein cardiac myosin-binding protein-C (cMyBP-C). cMyBP-C is critical for both normal systolic contraction and complete relaxation in diastole^[15-21]. However, the precise molecular mechanisms at play in *MYBPC3* mutations, including those that result in impaired muscle function leading to HCM, SCD, and HF, remain to be elucidated.

HCM has been considered a heterogeneous cardiac disease with incomplete penetrance and its variable phenotypic presentation, clinical manifestation, genetic etiology, age of onset, and severity. As they age, carriers are at considerable risk of developing significant clinical symptoms. Among all genes, mutations in *MYBPC3* are associated with late-onset HCM^[22]. The first clinical manifestation seen in carriers of *MYBPC3* mutations is a mild to moderate phenotype^[23-26]. Therefore, it is essential to determine the molecular mechanisms that underlie pathogenic phenotype in late onset and aging. Accordingly, this review aims to shed light on the contribution of age to HCM phenotype and examine the molecular mechanisms that drive age-dependent HCM penetrance in *MYBPC3* gene carriers.

PHYSIOLOGY, PATHOPHYSIOLOGY, AND CLINICAL MANIFESTATIONS OF HCM, A DISEASE OF THE SARCOMERE

HCM phenotype

HCM is characterized by thickening of the heart muscle, with or without left ventricular outflow obstruction, in association with diastolic dysfunction [Figure 1]^[5]. Sudden cardiac death is strongly linked to HCM, particularly in young adults and trained athletes^[6]. Clinically, HCM is defined by increased left ventricular wall thickness (end-diastolic left ventricular wall thickness ≥ 15 mm) or the equivalent relative to the body surface area in children^[4,23,25]. Diminished LV wall thickness (13-14 mm) is also considered a diagnostic feature if the patient is either genetically test-positive or has a family history of HCM^[27,28]. Hypertrophied myocardial fibers with disordered muscle bundles and interstitial fibrosis are additional typical diagnostic features. Additionally, luminal narrowing owing to high wall thickness in coronary microvasculature, myocardial ischemia injury, and fibrosis are observed^[5,27,29]. Diverse phenotypic expression and naturally variable progression of HCM are reflections of a range of clinical manifestations from dyspnea and/or syncope to sudden cardiac death. A subset of HCM also evolves into restrictive cardiomyopathy (RCM) or dilated cardiomyopathy (DCM) [Figure 1]. Based on its clinical features, HCM

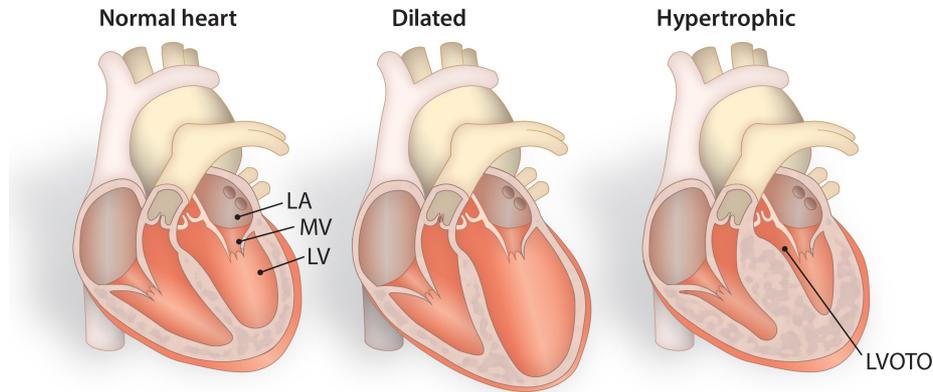


Figure 1. Schematic diagram differentiating normal hearts from dilated and hypertrophied hearts. Cardiomyopathies occur due to genetic variations, resulting in distinct physiological and/or pathophysiological consequences. In terms of clinical manifestations, cardiomyocytes within hypertrophied hearts become enlarged and demonstrate cardiac dysfunction due to increased left ventricular wall thickness, diminished left ventricular cavity size, and altered blood flow rates. LA-Left Atrium, MV-Mitral Valve, LV-Left Ventricle, LVOTO-Left Ventricular Outflow Tract Obstruction.

disease progression can be classified into subclinical HCM, classical HCM, adverse remodeling, and end-stage HCM or overt HCM with left ventricular ejection fraction (LVEF) falling below 50%^[11,27]. Most HCM cases exhibit a genetic mutation in one or multiple of the sarcomeric genes.

HCM is a disease of the sarcomeric genes

Extensive research has been conducted on gene mutations causing HCM. A primary focus of these studies is *MYH7*, a myosin heavy chain gene a major component of the thick filaments of the sarcomere. Further studies have been conducted on other sarcomeric genes, including *MYBPC3*, cardiac troponin T (*TNNT2*), tropomyosin 1 (*TPM1*), myosin light chain 2 (*MYL2*), myosin light chain (*MYL3*), troponin I (*TNNI3*), and α -actinin (*ACTC1*) [Figure 2]^[30]. Mutations in *MYH7* and *MYBPC3* genes are, however, the most frequent causes of HCM. Along with these sarcomeric proteins, regulatory proteins, such as calcium-handling proteins, are essential in maintaining sarcomere functions, but they also comprise a genetic subset of HCM disease phenotypes^[31]. About 30%-60% of HCM individuals are estimated to harbor sarcomere gene mutations^[32,33]. However, the expression of disease typically arises from a complex interplay of multiple mutations or a blend of causal genes and other factors, such as regulatory genes, epigenetics, and environmental influences^[31]. Similarly, pathogenic sarcomere variants exacerbate existing conditions, such as ventricular arrhythmia, HF, atrial fibrillation (AF), stroke, or even death in HCM individuals^[32,34]. Among the 50 unique cardiomyopathy genes with truncating mutations, *MYBPC3*, *TNNT2*, and phospholamban (*PLN*) exhibited a high ratio of cases compared to reference populations for HCM^[35,36]. Genetic mutations altering cardiac function are mostly missense mutations and truncating mutations. However, *MYBPC3* truncating mutations (e.g., nonsense, frameshift, \pm 1,2 splice) are predominant among HCM probands^[35-38].

AGING AS A RISK FACTOR FOR HCM

Phenotypic expression of HCM is age-dependent^[39] and presents unique clinical features with various causative genes and phenotypic variations from mild to severe symptoms^[40]. HCM-related crude mortality rates (CMR) stratified by age groups during the study period from 1999 to 2019 demonstrate that HCM mortality rates among U.S. residents were highest among those aged 75 and above. The crude mortality rate decreased for other age groups (15-29 years, 30-44 years, 45-59 years) from 2010 to 2019. However, no significant change was reported in the CMR of HCM patients aged 60 to 74 and over 75^[41]. A follow-up study from the Sarcomeric Human Cardiomyopathy Registry observed higher mortality rates owing to

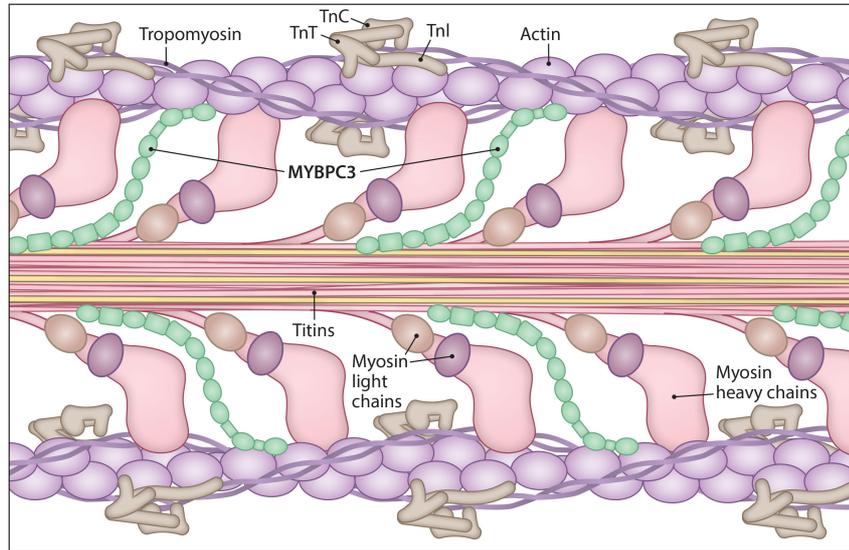


Figure 2. Structure and arrangements of myofilament protein in C-zone of cardiac sarcomere. The myofilaments within the sarcomere consist of two types: thick and thin filaments. The thick filaments are primarily composed of myosin protein. Each myosin molecule consists of a heavy chain that forms a tail and terminates with a globular head. Additionally, light chains interact with these globular heads. The giant protein titin spans across the sarcomere and is surrounded by myosin. The thin filaments are constituted by actin, forming a helical structure that interacts with the myosin globular heads. Cardiac myosin binding protein-C (*MYBPC3*), the cardiac paralog, is a regulatory protein that associates with actin and myosin, binding at its N-terminal and interacting with titin at its C-terminal. The thin filament protein, tropomyosin, wraps around the actin helix, and the three complex subunits of other thin filament proteins, known as cardiac troponins, namely Troponin C (TnC), Troponin T (TnT), and Troponin I (TnI), are also present.

HCM compared to the general U.S. population of similar age^[34]. However, this mortality rate was still highest for those 60 to 69 years of age. Otherwise, the risk of adverse events, such as ventricular arrhythmia, HF, AF, stroke, or death, was highest for individuals diagnosed with HCM before the age of 40, suggesting a milder effect of these events in the elderly compared to younger individuals^[32,34]. However, the left ventricle (LV) end-diastolic diameter and atrial diameter are larger in the elderly with more mitral valve calcification. Although SCD is common among the young or adolescents, clinical presentation of LV remodeling, LV dilation, and HF are more common in midlife and beyond, statistically strengthening the existing assertion that age is a strong influencing factor for HCM presentation^[31]. Apart from age, incomplete penetrance is also influenced by sex since women have a greater propensity to develop late-onset HCM^[41-43], and previous reports have shown that females with a pronounced HCM presentation had a poor prognosis^[44]. Although estrogen has cardioprotective effects, women may be more susceptible to diastolic dysfunction, which is the earliest sign of HCM^[45]. Reducing levels of estrogen during the transition phase of menopause might be a plausible explanation for the increased risk of women developing HCM 6 to 13 years later in life compared to men^[46].

In addition to clinical features, genetic screening for the sarcomeric gene was likely to be negative in elderly HCM patients^[47]. In a large cohort of 488 HCM probands, DNA was analyzed for mutations in the protein-coding exons of 8 common sarcomere genes (*MYBPC3*, *MYH7*, *MYL2*, *MYL3*, *TNNT2*, *TNNI3*, *TPM1*, and *ACTC1*)^[47]. Only 22% of HCM patients diagnosed after the age of 45 years had an identifiable mutation, whereas 49% of patients diagnosed before the age of 45 years had a mutation in the same set of genes^[31,48]. Thus, these population studies seem to forecast the aging process as a major influencing factor for HCM, irrespective of genotype. At the same time, early genetic testing allows more treatment options for genotype-positive individuals. The clinical features observed in genotype-positive and -negative individuals are heterogeneous, but age is a common contributor to the late onset of cardiac dysfunction. To account for

and understand the molecular events underlying the etiology of HCM, we hereinafter only consider the key biochemical cascades triggered by either gene mutation or age. Systematic reviews published on the genetic basis and the role of aging in HCM are excellent resources for gaining further perspectives^[49,50].

Molecular determinants of HCM

In HCM, cardiomyocyte hypertrophy is a major histological feature that results from a variety of pathways, including stress-sensing signaling pathways, the expression of trophic and mitotic genes, such as TGFβ1 and its downstream targets, the classical mitogen-activated protein kinase (MAPK) pathway, the phosphoinositide 3-kinase (PI3K) pathway, and the calcineurin pathway^[49]. However, in addition to genetic mutations, environmental and epigenetic factors also act as upstream modulators of these signal transduction pathways to initiate hypertrophy-related events^[51]. Epigenetic factors are inheritable changes that cause gene silencing by DNA methylation, histone deacetylation, and microRNAs (miRNAs)^[51-53]. Another crucial epigenetic mechanism, histone modification, has also been suspected of posing a risk for cardiovascular diseases. For example, histone acetyltransferase (HAT) p300 is upregulated in heart diseases^[54], and inhibition of histone deacetylase 2 (HDAC2) induces a hypertrophic signal^[55]. The contribution of DNA methylation and its associated molecules in the hypertrophic phenotype of cardiomyocytes is still undetermined. In the past few decades, post-transcriptional gene silencing of cardiac proteins by miRNAs has been extensively explored and found to be associated with myocardial infarction, arrhythmogenic cardiomyopathy, Long QT syndrome, and cardiomyopathies. The role of miRNAs in cardiac diseases and cardiac hypertrophy is not within the scope of the current review and miRNAs with presumed significance in cardiomyopathies are listed in [Table 1].

Molecular pathways in aging and the cardiac aging process

In most cases, the cause of HCM is inherited, while age-dependent penetrance is influenced by the variability in cellular responses to specific signals or stimuli. As just discussed, epigenetic factors are key effectors of phenotype in genetic diseases. The influence of aging on these epigenetic factors is addressed next in this review.

Aging/senescence

Aging, or cellular senescence, is a natural process whereby cells undergo alterations in morphology and function that, in turn, affect tissues, organs, and organisms. This process is characterized by reduced proliferation and regeneration capacity and increased cell death. Researchers have used long-lived mutants and mammalian animal models to understand aging-associated mechanisms. Senescence in cardiomyocytes involves increased expression of cell cycle inhibitors, proinflammatory cytokines, and senescence-associated β-galactosidase activity. Both mTORC1 and SIRT1 are primary molecules identified as regulators of lifespan^[56]. Other hallmarks include oxidative stress, oncogene overexpression, caloric restriction, reactive oxygen species, DNA damage, protein homeostasis, shortened telomere structure, inflammation, and autophagy [Figure 3]. Current next-generation sequencing platforms help visualize the epigenomic profile of aging, revealing histone methylation as having a strong association with aging^[51,57-60].

Aging and cardiac function

Cardiac aging is a progressive decrease in ventricular function and increased ventricular and arterial stiffness accompanied by fibrosis stimulated by angiotensin II and proinflammatory cytokines. Key signaling pathways, including mitochondrial adaptor p66shc, AMP-activated kinase, sirtuins, insulin/insulin-like growth factor-1 signaling, and cAMP, are crucial in coordinating senescence, metabolic changes, and cardiovascular phenotypes [Figure 4]. SIRT1, a nicotinamide adenine dinucleotide (NAD)-dependent deacetylase, is present at elevated levels in a healthy heart. However, aging leads to the downregulation and reduced nucleocytoplasmic shuttling of SIRT1 in the myocardium, resulting in

Table 1. List of miRNAs deregulated in MYBPC3 variant samples and their association with cardiac disease phenotype and aging

S. No	miRNA	MicroRNA expressions in HCM	Age/senescence/longevity-related changes in microRNA expression
1	miR-377-3p	Peripheral blood miRNAs-upregulated in HCM patients c.3369_3370insC and c.3624delC MYBPC3 mutation ^[143]	Upregulated in late passaged human skin ^[272] ; miRNA inhibition reduces senescence and increases cell viability ^[273]
2	miR-103a-3p		Lower in peripheral mononuclear cells of older individuals ^[203]
3	miR-146b-3p		Upregulated during senescence ^[274] ; lower expression levels in bronchial tissues of elderly ^[275] and aged macrophages ^[276] ; downregulated in perivascular adipose tissue of aged mice with cold stimulus ^[277] ; upregulated in aging heart ^[278]
4	miR-208b		Upregulated in young mice compared to older ones under post-traumatic osteoarthritis ^[279]
5	miR-200c		Upregulated in aging liver, aged skin ^[280,281] ; upregulation in endothelial cell death and senescence
6	miR-181-5p	Cardiac miRNAs-upregulated in cardiac tissues of HCM patients with c.927-2A>G, c.2373insG mutations ^[142]	NK cell aging ^[282,283] , age-related loss of muscle cells ^[284] , and skeletal cell aging ^[285] ; downregulated in peripheral blood and associated with all-cause mortality and age-related traits ^[286]
7	miR-184		Downregulated in calorie-restricted mouse brain ^[287] , older adults with major depressive disorder ^[288] , downregulated in aging heart ^[278]
8	miR-222-5p		Altered copy number in aging ^[289]
9	miR-96		Increased with age in peripheral blood mononuclear cells ^[290]
10	miR-204		Upregulated in aged hippocampus ^[291]
11	miR-10a	Cardiac miRNAs-upregulated in cardiac tissues of HCM patients with c.927-2A>G, c.2373insG mutations ^[142]	miR-10b, decreased in PD and increased in HD; both are age-associated diseases ^[292,293] ; downregulated in liver that senesces with aging ^[294]
12	miR-10b		MiR-10a*/miR-10-3p enriched in exosomes derived from frail, older individuals ^[295]
13	miR-652-3p	Myocardial miRNA upregulated in HCM ^[296]	Upregulated in aging heart ^[278]
14	miR-17-5p	HCM cardiac tissue upregulated miRNA ^[297]	Upregulated in aging heart ^[278]
15	miR-29a	Plasma miRNAs upregulated in HCM ^[298,299]	Upregulated in aging heart ^[300,301]
16	miR-1-3p	Downregulated in Left ventricular heart tissues ^[302] and HCM	Elevated levels in colon tissues of older individuals ^[303]
17	miR-27a		Upregulated in aging heart ^[278]
18	miR-155	Plasma miRNA downregulated ^[298] ; upregulated ^[302] in HCM/DCM	Lower in older individuals ^[203]

progressive cardiac dysfunction. Also playing a critical role in aging is insulin/insulin growth factor-1 signaling, which, when downregulated, increases lifespan^[61]. Moreover, suppression of PI3K and its downstream effector mTOR improves protein turnover and prevents the accumulation of lipofuscin, an age pigment^[51,62-64].

A whole genome transcriptome and proteome microarray was performed to profile age-related gene expression differences in heart tissues. It revealed that inflammation-related genes were elevated, whereas genes involved in protein homeostasis, such as protein folding and the ubiquitin cycle, decreased with aging^[65]. These intrinsic changes in cardiac systems increase the chance of pathogenesis of cardiovascular complications as age advances. From the perspective of heart dysfunction, apart from cardiac aging, pathogenic mechanisms in noncardiac tissues affected by aging also introduce additional risk factors, such as hypertension and diabetes. These factors contribute to the exponential increase in heart failure patients with advancing age^[66]. Additionally, autophagy^[67], telomere shortening^[68,69], and DNA damage response^[70,71] act as molecular triggers of cardiac hypertrophy. Furthermore, a set of autophagy-related genes (ATG) regulates various types of autophagy, including macroautophagy, microautophagy, and chaperone-mediated autophagy, which are defined by their role in longevity or lifespan^[72]. Among these genes, ATG5 has been linked to hypertrophy; cardiac-specific deletion of ATG5 resulted in hypertrophy, cardiac dilation, and

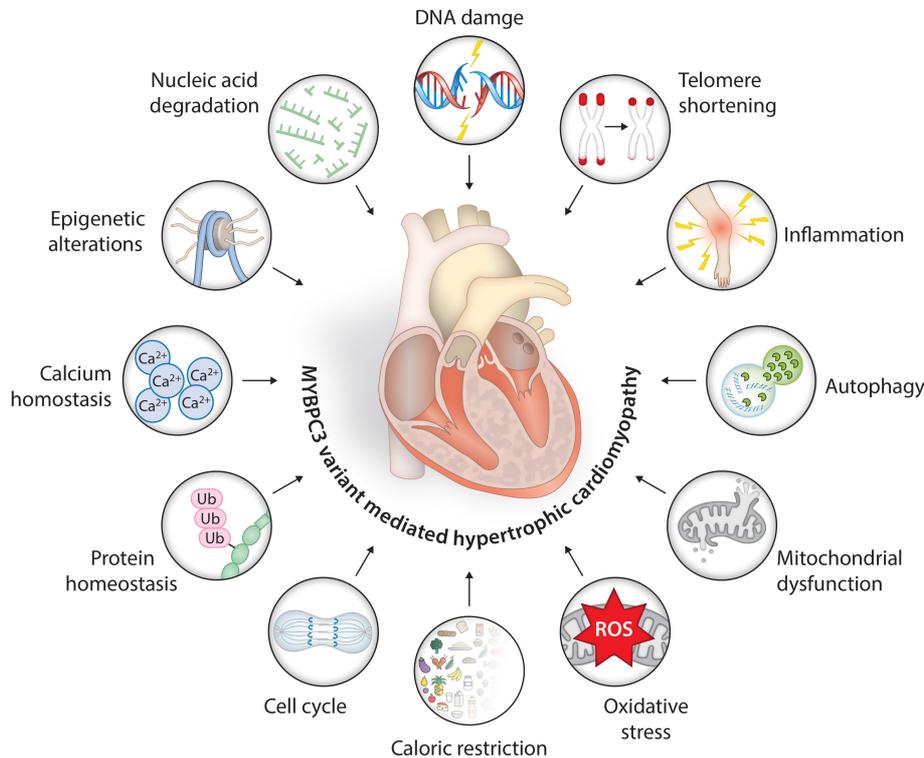


Figure 3. Schematic representation of *MYBPC3* gene variant-associated molecular events and age-associated hallmarks triggering hypertrophic signals in cardiac muscles. The nonsense-mediated mRNA degradation, ubiquitin proteasome-mediated protein degradation, alternate splicing, protein phosphorylation, and deregulated calcium sensing are the most common events in the HCM phenotype with *MYBPC3* mutations. The hallmarks of aging - namely, genomic instability, inflammation, autophagy, mitochondrial dysfunction, deregulated nutrient sensing, altered cellular senescence, protein homeostasis, and epigenetic alterations - activate aging-associated pathways in cardiac muscle cells. Both genetic mutation and aging-associated events generate pro-hypertrophic signals and induce hypertrophic phenotype affecting the cardiac muscle cell structure and function.

contractile dysfunction, along with disorganized mitochondria, abnormal sarcomere structure, and accumulation of misfolded protein^[73].

In summary, the collective evidence listed above indicates that genetic defects in the sarcomere cause sarcomere dysfunction and disarray, which in turn lead to cardiac fibrosis, dysfunction, and LVH. Additionally, environmental factors, metabolic syndrome, and aging contribute to a diverse range of penetrance effects and the late onset of HCM. Pathways and molecular events associated with hypertrophy and aging processes exhibit shared characteristics. This is significant because the chronological or biological age of an individual could serve as a triggering factor for HCM, influencing the onset of disease phenotype, either early or late in life.

***MYBPC3* GENOTYPE AND AGE-DEPENDENT INCOMPLETE HCM PENETRANCE**

Mutations in the *MYBPC3* comprise approximately 50% to 60% of all mutations that have been identified in patients with HCM^[14,74-77]. *MYBPC3* is the cardiac isoform (1273aa), which spans a 21kbp region with 35 exons. The other two skeletal isoforms, slow skeletal myosin binding protein-C (*MYBPC1*) and fast skeletal myosin binding protein-C (*MYBPC2*), share standard structural features, i.e., seven immunoglobulin domains and three fibronectin type III domains called C0-10 with myosin binding protein motif (M-domain) localized between C1 and C2^[78]. The amino terminal ends C0, C1, and C2 and the M-

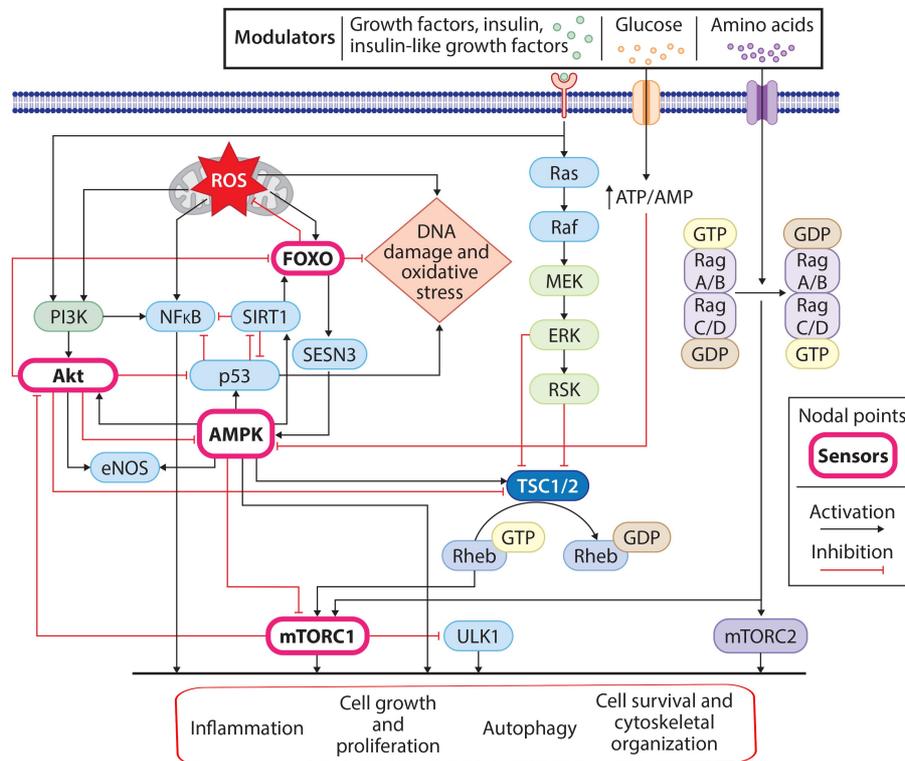


Figure 4. Key regulatory pathways involved in the aging process. Extracellular signals (nutrients, growth factors) and intracellular signals (genomic instability, mitochondrial dysfunction, and oxidative stress) initiate aging-associated signaling events. Cellular glucose uptake acts through the AMPK pathway, while growth factors, insulin, and insulin-like growth factors bind to their respective receptors and transmit signals through the Ras and PI3K-AKT pathways. Similarly, intracellular oxidative stress (ROS) and DNA damage-associated stress signals activate the PI3K, NFκB, SIRT1, and FOXO pathways. These pathways, either directly or through mTORC1, modulate cellular processes (elevated inflammation, reduced cell growth and proliferation rate, and inhibition of autophagy), leading to the aging phenotype.

domain can bind to F actin and S2 head regions of myosin heavy chain^[79,80]. The carboxy-terminal C7-C10 binds to the C-Zone of myosin thick filament^[81]. Interactions among titin, light meromyosin (LMM), and cMyBP-C occur in the C9-C10 region and C10 region, respectively [Figure 2]^[82]. Additionally, cMyBP-C and myosin are arranged alternately, connecting thick and thin filaments. cMyBP-C is critical for regulating actomyosin function and cardiac contraction^[83-86]. cMyBP-C acts as a brake by modulating cross-bridge kinetics, as determined by its phosphorylation status^[14,87]. Approximately 75% of the mutations in the *MYBPC3* gene are truncating mutations, which include nonsense, frameshift (leading to insertions or deletions), and splicing (including branch point) mutations. The C'-truncated cMyBP-C protein lacks myosin LMM- and/or titin-binding sites in the C-terminus region, leading to its failure to bind with myosin and titin and incorporate into the sarcomere^[88,89]. Nontruncated pathogenic variants, accounting for 25% of *MYBPC3* pathogenic variants, exhibit phenotypic effects like those of truncated variants. *MYBPC3* variants identified in some countries are population-specific and are classified as founder mutations, contributing to more HCM cases in the respective geographic location^[14]. *MYBPC3* variants result in mild to moderate phenotypes with late disease onset^[48,90-93]. The mechanisms underlying the late onset and development of HCM in *MYBPC3* carriers are still unclear.

MYBPC3 variants in elderly HCM patients

MYBPC3 variants are consistently associated with clinical manifestations of HCM. The symptoms are more prominent in older individuals carrying *MYBPC3* mutants owing to incomplete penetrance. Among the sarcomere gene variants identified, *MYBPC3* mutations have been associated with delayed expression of HCM and favorable prognosis^[94]. Often, HCM individuals with heterozygous *MYBPC3* loci have late disease onset with a non-threatening disease progression^[14]. In addition, *MYBPC3* variants may display a DCM phenotype in a few cases, such as a 25bp deletion in intron 32 of *MYBPC3*^[90]. A recent computational assessment of 73 non-truncating *MYBPC3* variants of uncertain significance predicted that 15 could affect RNA splicing, among which a few were experimentally confirmed using a minigene assay^[95]. Considering the pathogenic potential of *MYBPC3* mutations and their significance in HCM, most population screening results estimate that 40%-60% represent genotype-negative individuals^[37,96]. Recent studies reporting on cryptic gene variants of *MYBPC3* recommend including parallel gene sequencing for the entire *MYBPC3* region. Such parallel sequencing would include intronic gene variants and highlight the limitation of excluding splice region defects in current diagnostic HCM gene panels^[97,98].

HCM in elderly persons is genetically different from HCM in the unselected population^[31]. An analysis of individuals 63 ± 11 years of age with late-onset HCM found that most harbored *MYBPC3* mutations, implicating these variants as the common factor underlying age-dependent HCM^[31,99]. Yet another detailed familial study provided evidence for genotype-positive younger individuals with normal LV wall thickness; however, they had elevated wall thickness closer to midlife^[100]. Another study concluded that *MYBPC3* mutation is more prevalent in younger people compared to the chronologically aged at the time of genetic testing. However, the genotype-positive elderly were significantly affected by the presentation of atrial fibrillation, systemic hypertension, and greater left ventricular dimensions compared with younger individuals. Furthermore, the crescent-shaped LV in genotype-positive elderly patients suggested the role of *MYBPC3* mutation as a true cause of late-onset HCM^[101]. Many studies covering various geographic regions have defined the phenomenon of age-dependent incomplete penetrance in HCM gene carriers. These studies also attempted to establish the familial inheritance pattern by screening relatives. HCM and, in general, *MYBPC3* mutations are associated with later average age onset of symptoms, lower incidence of SCD, and benign clinical course. A few case studies emphasizing phenotype-negative diagnosis and age-dependent clinical outcomes, as highlighted in [Table 2](#), suggest age-dependent penetrance among *MYBPC3* carriers.

To further define age as a factor contributing to HCM presentation in *MYBPC3* variants, heterozygous mouse models of familial hypertrophic cardiomyopathy (FHC) were generated with *Myh7* missense mutation and *Mybpc3* truncation mutation. These animals developed progressive hypertrophy in cardiac muscles, with *Myh7* heterozygous mice developing onsets as early as 30 weeks and exhibiting shorter life expectancies. In contrast, *Mybpc3* heterozygous mice developed prominent differences in cardiac biomarkers, but only after 125 weeks of age, a pattern similar to that observed in human carriers^[102]. A novel isoform of mouse cardiac *Mybpc3* mutation has an additional 30 nucleotides that alter the splicing mechanism to generate mutant proteins. These mutant proteins created a disarray in the sarcomere structure, whereas their mRNA levels were predominantly expressed in the atria of aged mice, further implicating morphological and functional changes of cardiomyocytes during aging, i.e., age-dependent variation of cMyBP-C expression^[103]. These studies provide strong evidence that gene variants in *MYBPC3* can worsen the clinical course of HCM with advanced age.

MOLECULAR MECHANISMS UNDERLYING THE DEVELOPMENT OF HCM IN *MYBPC3* VARIANTS

The mechanisms behind the late onset and development of HCM in *MYBPC3* gene carriers remain unclear; nonetheless, the pathogenesis of familial HCM has been the focus of every mechanistic tier, from genotype to phenotype^[49]. The two main mechanisms controlling the pathogenicity of *MYBPC3* gene variants are allelic imbalance and haploinsufficiency^[104,105]. Allelic imbalance refers to a disproportion in wild-type and mutant allelic gene transcripts. In haploinsufficiency, when one copy of a gene is deleted or contains a loss-of-function mutation, the dosage of normal product generated by the single wild-type gene is not sufficient for complete function. It should be noted that translated wild-type protein is insufficient to meet the cellular requirement in cases of haploinsufficiency^[35]. Epigenetic alterations can also play a significant role in developing hypertrophied cardiomyocytes^[53,106,107]. In addition, recent studies provide evidence for the intercellular variation of myofibrillar cMyBP-C expression within the myocardium from HCM patients with heterozygous *MYBPC3* mutations^[108]. Furthermore, alteration or mutations in the regulatory components, including transcription factors, miRNAs, splicing-associated proteins, proteins of nonsense-mediated decay (NMD), autophagy and the ubiquitin-proteasome system (UPS), as well as genotype-dependent differential expression of regulatory proteins, could also present a path forward in studying the molecular mechanisms underlying late onset of HCM in *MYBPC3* carriers [Table 3]. Therefore, continued intensive investigation of these pathways could finally reveal the molecular basis of genetic HCM.

Transcription-dependent mechanisms

Transcription has been poorly studied for *MYBPC3*; however, computational prediction of the ~4 kb upstream promoter region of *MYBPC3* revealed potential binding sites for GATA and MEF2 transcription factors^[109]. According to Akazawa and Komuro^[110], cardiac transcription factors have been directly associated with regulating cardiac sarcomeric genes and are involved in the development of cardiac hypertrophy, which is significant in cardiac muscle development and regulates cardiac specificity^[111,112]. Differences in wild-type and mutant *MYBPC3* mRNA levels can be attributed to alterations in the interactions between trans-acting factors and cis-regulatory regions, either due to varying levels of transcription factors or epigenetic modifications at the *MYBPC3* promoter region [Figure 5A]. For instance, reduced *MYBPC3* mRNA levels were found in six genotype-positive HCM individuals with specific *MYBPC3* mutations compared to genotype-negative HCM individuals, suggesting a potential transcriptional regulation of *MYBPC3*^[110]. Analysis in *MYBPC3* null mice revealed the involvement of Zinc finger and BTB domain containing 16 (ZBTB16) and other genes of HCM significance^[113]. The role of GATA, MEF2, and ZBTB16 in *MYBPC3* expression remains unclear. However, multi-omics analysis in *MYBPC3* carriers identified several transcription factor binding motifs in hyper- and hypoacetylated regions, which need further confirmation for their role in *MYBPC3* transcription^[114]. Additionally, increased methylation of *MYBPC3* in comparison to its skeletal isoform explains the genetic instability and increased occurrence of mutation in *MYBPC3*^[115]. Although these epigenetic and genetic factors might explain the differential transcription level, their role in allelic imbalance needs to be verified. In line with the aim of this review, integrated transcriptomic and proteomic analyses might shed more light on age-related HCM in *MYBPC3* carriers.

Post-transcriptional dependent regulation

Post-transcriptional regulation mainly involves RNA-binding proteins and non-coding RNAs, such as microRNAs, piwi RNAs, and long non-coding RNAs. These factors play roles in RNA processing, export, localization, turnover, and translation^[116,117]. Among these, post-transcriptional control mediated by alternative splicing (AS), NMD, and post-transcriptional gene silencing (PTGS) in *MYBPC3* mutations is investigated next.

Table 2. MYBPC3 genetic mutations familial case studies presenting age-dependent penetrance

MYBPC3 mutation	No. of gene carriers	Age of probands during screening and their clinical presentation
c.2833_2834del, p.Arg945fs	4 of 176 HCM probands, 1 of 54 DCM probands, 1 relative of these 5 probands	61 ± 10 years (48-79 years); LV systolic dysfunction and suffer from cardiovascular events midlife and beyond ^[304]
c.2373dup, p.Trp792fs	27 of 49 probands; only 10 were symptomatic, 5 with borderline, and 12 asymptomatic	29-69 years; symptomatic HCM underscored by more advanced age of 54.7 ± 4.5 years; borderline HCM or unaffected carriers were 40 ± 14.0; non-gene carriers were 43 ± 12.9; asymptomatic at 37.8 ± 13.6 ^[137]
c.2459G>A, p.Arg820Gln	8 probands (7 HCM, 1 DCM) of 250 HCM probands and 90 DCM probands. Among 24 relatives of these 8 probands, 17 were genotype-positive	16-77 years; burnout phase of HCM (patients with over dysfunction defined by an LVEF < 50% ^[11]) was described in those > 70 years of age. The disease penetrance was 70% in subjects > 50 years of age by echocardiography and 100% by ECG, and in those aged < 50 years, it was 40% and 50%, respectively ^[305]
c.1777del, p.Ser593fs, (V592fs/8c)	15 of 94 probands, 24 relatives of these 15 probands were genotype-positive	48 ± 14 (16-83); 100% disease penetrance in ≥ 50 years; 65% disease penetrance among individuals < 50 years ^[92]
g.47332282_47332306del	49 (13.8%) carried the 25-bp deletion (46 heterozygotes and three homozygotes) of 354 cases	48 ± 8 (Group 1 case) and 49 ± 12 (Group 2 case). Symptomatic carriers 55.7 ± 15.3 years; asymptomatic carriers 40 ± 14.1 years among 120 family members of 28 affected families with cardiomyopathy; 90% of the oldest members are symptomatic, whereas young and middle-aged are asymptomatic ^[90]
c.1000G>A, p.Glu334Lys	9 of 1017 unrelated probands were gene carriers	15-76 years; Delayed clinical presentation, reduced penetrance of HCM in women than men ^[306]
c.2308 + 1 G>A, p.I108A _{lafs} *9	13 probands and their relatives of a total of 107 individuals were screened; among the 54 gene carriers, 39 had HCM	Male-50.5 ± 15.9, female-65.5 ± 17.4 years; lower penetrance rate and later onset in women than men ^[307]

Alternative splicing

Alternative splicing is a vital process in RNA maturation, involving the removal of introns and the joining of exons. Splicing factors such as Serine/arginine-rich splicing factors (SRSFs), heterogeneous nuclear ribonucleoproteins (hnRNPs), nova proteins, transformer2 (TRA2), CUG-BP and ETR-3-like factor (CELF) proteins, and RNA-binding proteins (RBPs) play critical roles^[118-120]. Splicing events lead to exon inclusion/skipping, intron retention, alternative splice site selection, and back splicing, generating circRNA molecules, stable and prevalent RNAs in eukaryotic cells that arise from back-splicing. Deregulation in alternative splicing events leading to the development of cardiac-related disease phenotypes has been recently reviewed^[121]. In the context of cardiomyopathies associated with *MYBPC3*, cardiac dysfunction predominantly arises from splicing abnormalities stemming from mutations at donor or acceptor sites or the insertion and deletion of intronic sequences. Among *MYBPC3* mutations, splice donor variants constitute 1% and splice acceptor variants constitute 2%^[88]. However, apart from conventional splice site mutations, screening of *MYBPC3* intronic variants identified four intronic variants in introns 9 and 13. These were predicted to cause cryptic splice sites in conventional splice site mutation-negative HCM patients. This signifies the importance of alternative splicing as a potential pathogenic mechanism in *MYBPC3* carriers [Figure 5B]^[98]. Additionally, genetic defects in SRSF, hnRNPs, and RBPs have been implicated in severe and complex cardiomyopathies^[122]. While defects in RBM20, a gene that encodes a protein that binds RNA and regulates splicing, have been identified as critical contributors to the development of both DCM and HCM, *MYBPC3* is not a substrate for it^[123,124]. However, RBPMS2 has been identified as a splicing protein for *MYBPC3* in zebrafish^[125]. Cardiomyocytes differentiated from human embryonic stem cells exhibited disrupted sarcomeric structures, the transcriptomic analysis of which revealed that *MYBPC3* was aberrantly spliced in RBM24 null phenotype^[126], explaining the role of these RBPs in processing *MYBPC3* mRNA.

Nonsense-mediated decay

Mature mRNAs transported to the cytoplasm with premature stop codons (PTCs) or transcripts lacking

Table 3. Aging effect on potential molecular regulators of MYBPC3 and HCM

Age/senescence/longevity-related changes	
Transcriptional regulators	
Transcription factors	
GATA	GATA4 accumulates in senescent cells ^[308] ; GATA6 expression was decreased in younger mice or later passaging of cultured mesenchymal stem cells ^[309]
MEF2	Age-dependent downregulation of MEF2 in microglial cells; rescues retinal explant culture from age-dependent photoreceptor degeneration ^[310]
KLF15	Downregulated in aged subcutaneous white adipose tissue ^[311,312]
ETS2 (ETV4)	Elevated levels of ETS2 promoted apoptosis-inducing factor-mediated programmed necrosis ^[313]
RXRA	Deficiency results in cellular senescence. RXR pathway genes decreased expression in aged macrophages in monocytes of multiple sclerosis patients. RXR receptors and other nuclear receptor factors regulate cellular senescence ^[314-316]
Post-transcriptional regulators	
Alternative splicing	
RBM24	Cardiomyopathy-related RBM24 regulated p53 expression, which is known to induce aging-related heart failure ^[194,195,317]
RBPM52	Age-associated decreased mRNA expression in primary dermal fibroblast ^[197,198]
SRSF3 and SRSF1	Cellular senescence and longevity ^[189-191]
Srfs7, Srsf2, Y box binding protein, 1 Hnrnpa0, Hnrnpa1, Hnrnpd1, Sfpq	Elevated expression in young mice ^[193]
NMD Pathway	
UPF1	SMG-2/UPF1 promotes longevity in worms (<i>C. elegans</i>) ^[199,318]
UPF3 (UPF3A and UPF3B)	Deletion of UPF3 decreased the mean chronological lifespan in yeast ^[319] . UPF3B is associated with age-related disorders ^[320]
Post-translational regulators	
Protein modification	
PKA	Mice lacking the PKA gene have extended lifespans and resistance to cardiac dysfunction ^[220,221]
PKB, JNK, CaMKII	Deregulated in animal models of aging ^[222-224]
Calpain	Calpains are activated in aging ^[321] . Calpain inhibitors improve/reverse age-associated damage in heart function ^[322,323]
Protein degradation	
Atrogin-1 (MAFbx) and MuRF1 (Trim63)	Differential expression in age-associated sarcopenia, muscle wasting, and diet restriction in rats and human muscle tissues ^[324-330] ; elevated levels of atrogin-1 reduce age-associated cardiac fibrosis ^[331] , and its deficiency promotes premature death, cardiomyopathy, and heart failure ^[332]
ATG-5	Cardiomyocyte-specific constitutive knockout of Atg5 enhanced susceptibility to pressure overload. Cardiac-specific Atg5 knockdown mice exhibited age-related cardiomyopathy. Podocyte-specific knockout caused spontaneous age-dependent late-onset glomerulosclerosis. ATG5 is downregulated in normal human brain aging. ATG5 transgenic mice are lean and have extended lifespans. Embryonic fibroblasts cultured from ATG5 transgenic mice are tolerant to oxidative stress and damage ^[73,333-336]
HSC70	Decreased LAMP-2A and Hsc70 protein levels in the muscle of aged mice remain unchanged in heart. Decrease in levels in olfactory bulbs ^[238,240]
BAG-3	Switching from BAG-1 to BAG-3 in aging BAG-3/BAG-1 ratio elevated in neurons during aging of rodent brain ^[237,239]

proper stop codons are degraded through nonsense-mediated decay (NMD) or non-stop decay mechanisms. The RNA surveillance system, which is initiated by the identification of premature termination codons, recruits several multiprotein complexes, including exon junction complexes composed of four core proteins and additional peripheral proteins that bind to 20-25 nucleotides upstream of the exon junction. Translation termination complex and release factors interact with up-frameshift factors (UPF), including UPF1, UPF2, UPF3A, and UPF3B. Phosphorylated UPF1 recruits the SMG-5/SMG-7 complex, triggering exo- and endonucleolytic degradation^[127]. An estimated 70% of *MYBPC3* mutations are truncated with a premature termination codon^[128]. Under experimental conditions, overexpression of C'-truncated

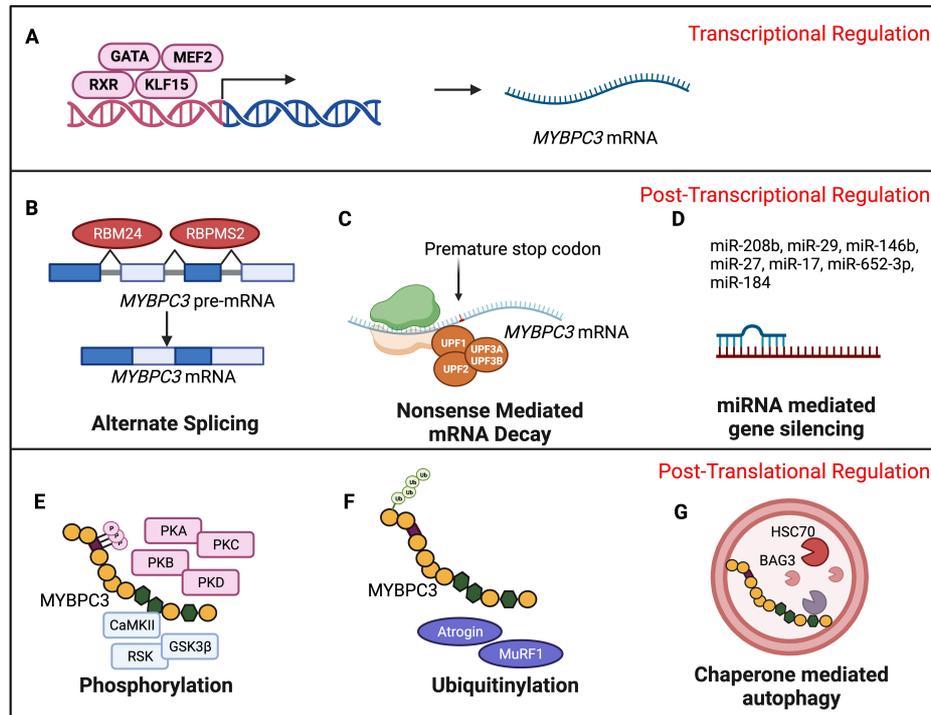


Figure 5. Potential HCM and aging-associated molecular events mediate the late onset of HCM among *MYBPC3* gene carriers. (A) Differential expression and enrichment of trans-acting factors in *MYBPC3* cis-elements regulate *MYBPC3* transcription upon aging. (B) Age-associated altered splicing mechanisms result in aberrant splice products, and (C) dysregulated mRNA degradation through nonsense-mediated pathways determines the abundance of *MYBPC3* mRNA available for translation. (D) Aging-induced differences in the expression of miRNAs might modulate *MYBPC3* translation through post-transcriptional gene silencing. (E) Age-dependent changes in phosphorylation, (F) ubiquitination proteasome system, (G) and chaperone-mediated autophagy determine the sufficiency of *MYBPC3* protein in preserving cardiac function (Figure Created with [BioRender.com](https://www.biorender.com)).

cMyBP-C in cultured cardiomyocytes^[129,130] and mouse models^[131-135] showed the incorporation of mutant cMyBP-C into the sarcomere. However, there was no evidence of the presence of C'-truncated cMyBP-C in human biopsies from HCM patients^[136,137]. Even though the NMD pathway acts as a primary pathway in degrading mutant mRNA in genetic diseases causing haploinsufficiency, mutant transcripts sometimes bypass the NMD pathway, resulting in the expression of truncated peptide causing poison polypeptide effects [Figure 5C]. In mice, *MYBPC3* mRNA with PTC mutations has cleared through the NMD pathway^[104,138]. To date, studies evaluating the role of NMD genes in the degradation of mutant *MYBPC3* mRNA are limited. NMD inhibitors, such as emetine, a translational inhibitor, or cycloheximide (CHX), another NMD inhibitor, indirectly showed that mutant *MYBPC3* mRNA are cleared through NMD and escape this surveillance upon NMD inhibition^[104]. Despite the difference in transcript levels, it has been recently discovered that protein levels were similar in cardiomyocytes cultured from isogenic lines of iPSCs derived from *MYBPC3* PTC variant p.R943x and its respective noncarriers^[139]. Transcriptome analysis of these isogenic lines revealed that NMD pathway genes were activated, whereas cardiac signaling pathway-related genes were dysregulated, which was reversed upon inhibition of NMD^[139]. However, a recent discovery identified UPF3B as a Z-disc-localizing protein involved in the degradation of truncated *MYBPC3* mRNA in left ventricular septum tissue from HCM patients carrying *MYBPC3* mutations (c.3288delG, c.2864_2865delCT, c.3697C>T, c.1458-6G>A, c1700_1701delAG, c.3490+1G> T, and c.927-2A>G)^[140]. The accumulation of mutant mRNA evidence provides greater insight into the involvement of NMD surveillance in degrading the *MYBPC3* RNA isoforms generated by various types of mutations. This calls for further exploration of different NMD components in late-onset and disease heterogeneity of HCM in *MYBPC3* carriers.

Post-transcriptional gene silencing-mediated regulation

Among the many regulatory pathways of mRNA, PTGS has provided functional insights into the non-coding regions of the genome. PTGS mainly involves translation inhibition or mRNA decay through the recruitment of RNA-induced silencing complex (RISC) upon binding of miRNA^[141]. No direct evidence can confirm the miRNA-mediated regulation of *MYBPC3* expression; however, independent studies observed differential miRNA expression in HCM individuals carrying *MYBPC3* mutations. The miRNA profiling of the endocardial interventricular septum regions of *MYBPC3* carriers with founder mutations c.927-2A>G (splice site mutation) and c.2373insG (truncation mutation) discovered that levels for 13 miRNAs were altered. Of these, 10 were upregulated (miR-181-a2*, miR-184, miR-497, miR-204, miR-222*, miR-96, miR-34b*, miR-383, miR-708 and miR-371-3p) and 3 were downregulated (miR-10b, miR-10a* and miR-10b*), suggesting crucial roles for PTGS in the initiation and progression of cardiac dysfunction^[142]. In a similar study, plasma miRNA profiling of *MYBPC3* carriers with c.3369_3370insC and c.3624delC mutations reported that 385 miRNAs were upregulated, 279 miRNAs were downregulated, and 288 miRNAs were unchanged. About 33 miRNAs significantly differed in their expression levels (28 upregulated and 5 downregulated) between HCM and control subjects, forming the basis for establishing the significance of miR-208-3p in HCM^[143]. These studies revealed notable alterations in miRNA profiles, thus highlighting the potential of miRNA-mediated pathways in understanding and potentially mitigating the complexities of genetically inherited diseases such as HCM [Table 1, Figure 5D].

Post-translational modification-dependent regulation

After RNA processing and surveillance, mRNAs undergo translation, forming nascent polypeptide chains that enter the protein trafficking system and fold into tertiary protein structures. Stable proteins undergo enzyme-catalyzed PTMs on their side chains or backbones, enhancing their function. PTMs, including phosphorylation, ubiquitination, SUMOylation, O-GlcNAcylation, methylation, and acetylation, have implications in cardiac hypertrophy. These PTMs, triggered by various stimuli, activate or deactivate enzymes and signaling molecules, contributing to protein homeostasis and leading to changes in protein diversity, localization, structure, and interactions with other molecules. In cardiomyocytes, PTMs compensate for weak spatiotemporal transcriptional regulation and play a critical role in the cardiac hypertrophic process through intricate mechanisms^[144,145].

Protein modification-related mechanisms

Based on function and localization, protein molecules can undergo any one of a variety of covalent modifications, such as phosphorylation, acylation, alkylation, glycosylation, and oxidation, each one catalyzed by dedicated PTM enzymes. Various PTMs and their role in cardiac hypertrophy have been recently reviewed in detail^[145]. Among these, protein phosphorylation by kinases constitutes a major PTM since most cellular processes rely on the phosphorylation status of its respective target protein. cMyBP-C, a thick-filament protein, exerts contractile function depending on its phosphorylation status^[84,146-150]. The cMyBP-C protein has several phosphorylation sites, and the number of sites varies among species. For instance, human cMyBP-C has four phosphorylation sites, mice have three or four, canines possess five, and rats have three, all previously characterized for their roles in HCM^[85]. An *in vivo* study identified N-terminal 17 residues confined to the N-terminal end of the protein (C0-C2) [Figure 2]^[151]. The phosphorylation of cMyBP-C among these sites was essential to rescue cMyBP-C null mice, implying its crucial role in disease pathogenesis^[146]. cMyBP-C phosphorylation sites are targets of protein kinase A (PKA; Serine 273, 282, 302, mouse sequence numbering)^[146,149], protein kinase C (PKC: Ser 273, 302)^[152-156], calmodulin-dependent kinase II (CaMKII; Ser 302)^[155], protein kinase D (PKD: Ser 302), and ribosomal S6 kinase (RSK; Ser 282). Additional phosphorylated sites Thr290 (mouse)^[157] and Ser 133 have been identified where Ser133 is

predicted to be the target of Glycogen Synthase Kinase 3 Beta (GSK3 β) [Figure 5E]^[158]. Phosphorylated cMyBP-C is protected from proteolysis, but dephosphorylation has been associated with failing hearts. In spite of the presented evidence, the phosphorylation status of cMyBP-C remained unchanged in HCM patients with frameshift mutations in *MYBPC3* compared to their respective donor controls^[105,159]. The contribution of cMyBP-C phosphorylation to late-onset HCM in gene carriers and the underlying mechanisms remain unclear. Apart from phosphorylation, acetylation of sites within a 40 kDa fragment of cMyBP-C was also determined to affect the contractility and function of the myofilament complex^[129,130]. Similarly, S-glutathionylation of cMyBP-C in the heart tissue of a hypertensive mouse model resulted in the development of diastolic dysfunction^[160]. The cMyBP-C protein is also subjected to calpain cleavage, citrullination, and s-nitrosylation^[161-163], all requiring further research to identify the significance of these mechanisms and their effects on HCM pathogenesis in *MYBPC3* carriers.

Protein degradation machinery

Protein levels undergo tight spatiotemporal regulation such that excess, unneeded protein or any misfolded and mutant proteins are targeted by the protein degradation system, either through the ubiquitin-proteasome system (UPS) or autophagy.

(1) UPS: Proteins destined for degradation are modified by UPS-specific enzymes, i.e., E1, E2, and E3 ligases, in a reversible enzymatic reaction using ubiquitin as a substrate. The tagged proteins undergo proteolysis with the help of 26S proteasomes^[164-168]. The second major HCM molecular mechanism observed among *MYBPC3* carriers was the dominant-negative phenotype owing to the generation of poison polypeptide^[133]. Here, truncated cMyBP-C can be incorporated into the myofilaments, destabilizing the sarcomere. This leads to altered energetic mechanisms, activation of profibrotic pathways, constant stress, and premature death of the cardiomyocytes^[133]. Further, in certain mutations, the lack of detectable mutant protein in heart tissue suggests that protein was not synthesized from mutant mRNA or was degraded rapidly, even if translated, eliminating the possibility of poison polypeptide-mediated pathway in the HCM phenotype^[169]. Using inhibitors, such as epoxomicin (selective 20S proteasome inhibitor)^[104], bafilomycin (autophagosome lysosome inhibitor), and lactacystin (UPS inhibitor)^[170], it is evident that the cMyBP-C mutant/truncated protein undergoes substantial UPS-mediated degradation [Figure 5F]. Among various E3 ligases, muscle-specific E3 ligases are involved in activating proteostasis in cardiomyocytes^[171]. Of note, atrogen-1/MAFbx (Z-disc) and the muscle ring finger proteins (MuRFs), including MuRF1-M band and MuRF-3-Z disc, are of particular interest by their localization in sarcomeres, and cMyBP-C acts as one of their substrates^[172]. While atrogen-1 or MuRF levels remain unchanged in HCM hearts of *Mybpc3* knock-in mice, Asb2 E3 ligase is downregulated^[173].

(2) Autophagy: This lysosomal-dependent protein degradation mechanism comprises macroautophagy, microautophagy, and chaperone-mediated autophagy^[174]. Contrary to UPS-mediated cMyBP-C degradation, coimmunoprecipitation and mass spectrometry revealed that the Heat shock protein(HSP)-70 (HSP) family of chaperones interact with wild-type and mutant *MYBPC3* protein, among which Heat shock cognate 71 kDa protein (Hsc70) was identified as an abundant interactor^[175]. Notably, HSC70 is primarily involved in chaperone-mediated autophagy, whereby substrate proteins are targeted for lysosomal degradation by cathepsins^[176]. The assembly of BAG-3 with HSC70 and HSPB8 to form a chaperone-selective autophagy (CASA) complex is crucial for cardiomyocyte contractility^[177]. CASA complex helps stabilize the myofibrillar structure and inhibits myofibrillar degeneration under mechanical stress conditions^[178]. BAG-3 expression was downregulated in DCM, and *MYBPC3* was one of eight myofilament proteins that interact with the CASA complex in the human heart^[177]. Thus, the autophagy process involving BAG-3 and HSC-70 through sarcomeric protein quality control mechanisms and structural stability related

mechanisms highlights the crucial role(s) of autophagy in the etiology of cardiomyopathy [Figure 5G]. Although these mechanisms have been investigated for several years, the role of protein degradation in the late onset of HCM in *MYBPC3* carriers remains unclear^[35].

ASSOCIATION OF *MYBPC3* VARIANTS, AGING AND THE SEVERITY OF HCM

A systematic compilation of age-associated molecular changes in cardiovascular function has been recently published^[50]. A close look at HCM pathogenetic mechanisms reveals that HCM processes are congruent with those of aging since they share some common hallmarks [Figure 3]. These include epigenetic changes, transcription factor activity, RNA processing and stability, transcriptional noise, and mitochondrial dysfunction. The major pathways in HCM with underlying causes of *MYBPC3* mutation include RNA metabolism, i.e., alternative splicing, NMD pathway, and protein metabolism (UPS). As discussed in the section below, age-associated aberrant splicing events, defective NMD, autophagy, and UPS systems might act as triggering events of HCM disease progression in *MYBPC3* carriers at older ages.

Aging and alternative splicing

The effects of aging on transcription and translation processes have been a primary research focus for many years. Recently, however, alternative splicing, an intermediate step, has also gained attention for its significance in the aging process. The key finding is that aging increases isoform variations across species and tissues. Many components of the RNA processing machinery are themselves regulated by splicing. The differential expression of splicing trans-acting factors, aberrant RNA splicing, and aging genes provides mechanistic insights into splicing in age-dependent disease phenotypes^[179]. Several studies have reported changes in splicing, e.g., reduction of unspliced transcripts and formation of more circular RNAs upon aging^[180-188]. Additionally, the deregulation in splicing of genes involved in age-associated pathways, such as NFκB, mTORC1, and AMPK, plays a major role in the aging phenotype. These can be mediated through increase or decrease of specific isoform function, and imbalance in isoform ratio. Similarly, in trans-acting factor dysregulation, splicing factor levels also contribute to aberrant yields of splice variants. Interestingly, serine- and arginine-rich splicing factors SRSF3 and SRSF1 were reported to be significant in cellular senescence and longevity^[189-191]. Alterations in splicing have been recognized in various tissues, and cardiac tissue-specific age-related changes in splicing factors have been reviewed in detail with a focus on cancer, neurodegenerative tissues, and progeria^[181].

More precisely, cardiac transcriptome and proteome analysis of young and early-aging mouse hearts determined widespread exon usage patterns compared to differential gene expression. The differential transcript levels of RNA-binding proteins and splicing factor-related genes were correlated with corresponding alterations in the levels of protein splice variants in the aging process^[192]. Similarly, transcriptomic analysis of juvenile mouse versus adult cardiomyocytes, hepatocytes, and cerebral cortex found a higher expression of splicing factors, such as serine- and arginine-rich splicing factors (Srsf7, Srsf2), Y-box binding protein 1, heterogeneous nuclear ribonucleoproteins (Hnrnpa0, Hnrnpa1, Hnrnpdl) and splicing factor proline- and glutamine-rich (SFPQ) protein, in young mice^[193]. Since alternative splicing is one of the molecular mechanisms of HCM in *MYBPC3* splice site variants^[128], age-related changes in alternative splicing events and splicing factors, as explained above, might contribute to the severity of HCM disease phenotypes. Cardiomyopathy-related RBM24 regulated p53 expression, which is known to induce aging-related heart failure^[194,195]. Similarly, methylation-mediated RBPMS2 downregulation has been established^[196], and down-regulation of its mRNA in primary dermal fibroblasts has been associated with aging^[197,198]. By elucidating the levels of RBM24^[126] and RBPMS2, we will be able to identify *de novo* pathophysiological mechanisms in age-associated HCM phenotypes.

Aging and NMD pathway

The NMD pathway is key for maintaining RNA quality, but its role in the aging process is not yet well explored. Longevity mutants of animal models (*C. elegans* Insulin/IGF-1 receptor *daf-2* mutant) help in understanding this phenomenon in the process of aging. In this model, silencing of NMD components suppressed longevity, showing that NMD protects the cells from undesirable proteins through its quality control. Both RNA-Seq analysis and mRNA half-life experiments suggested that enhanced NMD activity would prolong the longevity of the mRNA stability and translation. Overall, the reduction of the NMD pathway might hasten the aging process^[199]. In addition, genetic analysis of RNAi feeding clones with lengthened lifespans identified *smg-1* inactivation as a key factor in increasing the longevity of those clones. Interestingly, *smg-1* is known to play a conserved role in the NMD pathway of worms and mammals^[200]. The requirement of the NMD pathway has been implicated in the transition of cells from embryonic lineage towards differentiation. Hutch *et al.* identified an intricate connection between mRNA homeostasis and mTORC1 activity^[201]. They show that NMD deficiency increased mTORC1 activity via the production of a premature termination codon (PTC) isoform of elongation initiation factor^[201]. The mTORC1 is a key molecule in autophagy- and aging-related pathways, and its modulation through NMD clearly explains the involvement of the NMD pathway in lifespan. The involvement of mRNA decay in cardiac aging remains unknown. As previously mentioned in section 5.2.2, PTC mutation activates the NMD pathway^[130], whereas specific inhibition of the NMD pathway reverses the molecular phenotype and calcium handling abnormalities^[139]. This might not be an effective treatment for the late onset of HCM, as the NMD pathway needs to be preserved to enhance the RNA surveillance mechanism during aging. However, further extension of research on animal models of aging will be crucial to determine the role of the NMD pathway and the RNA surveillance system in HCM disease with PTC mutations^[139]. Thus, given a scenario wherein reduced NMD activity increases the senescence-associated phenotype, aging-associated elevated levels of mRNA and protein with *MYBPC3* PTC mutations might exacerbate HCM-related molecular changes. In the absence of scientific consensus, further exploration of NMD pathway dysregulation in the context of cardiovascular aging and its molecular consequences in genetic PTC mutations in *MYBPC3* carriers will shed more light on its significance in late-onset HCM.

Aging and PTGS

Several studies using animal models and longevity experiments discovered that miRNAs are, indeed, a determinant of the aging process^[202-206]. HCM miRNAs, as listed in [Table 1](#), have been implicated in aging/elderly phenotypes^[207]. A detailed review of key miRNAs involved in aging and cardiac aging is found elsewhere^[205,208-210]. No evidence can be found of miRNA-mediated post-transcriptional gene silencing of *MYBPC3*; however, microarray of heart tissue samples and RNA sequencing of blood miRNAs have left us with few candidate miRNAs that are differentially expressed in HCM patients carrying *MYBPC3* variants. Among these identified miRNAs, miR-377, miR-200c, miR-208b, miR-103, miR-181-5p, miR-184, miR-96, miR-34, miR-383, miR-708, and miR-10 are all differentially expressed in the aged phenotype, such as the late passage cells, aged animal models and older individuals. Their involvement in regulating cellular senescence and longevity mechanisms has been implicated in different tissues [[Table 1](#)]. Further validation of these miRNAs in heart tissues of aged cells/animals or human disease models will help uncover novel roles for miRNA-mediated HCM and its age-dependent HCM penetrance in *MYBPC3* carriers.

Aging and post-translational regulation of cMyBP-C

Chronic α -adrenergic receptor (α -AR) stimulation can result in the development of left ventricular hypertrophy and HF^[211]. Novel therapeutic strategies, such as direct activation^[212,213] and/or inactivation^[214] of myosin, are needed to ameliorate the side effects associated with current pharmacological therapies. α -AR stimulation activates protein kinase A, which phosphorylates sarcomeric myofilament proteins, including cMyBP-C. cMyBP-C undergoes other post-translational modifications (PTM), including ubiquitination,

SUMOylation, O-GlcNAcylation, methylation, carbonylation, and acetylation^[215,216]. However, a systematic study is required to link the PTM of cMyBP-C to the development of HCM^[217-219].

Post-translational modification and aging

cMyBP-C phosphorylation is a significant phenomenon in age-dependent incomplete penetrance of HCM^[147]. Phosphorylation and PTMs affect cardiac function^[85] and the status of PTM processing, along with the activity of various enzymes involved in these modifications upon aging, might be predictors of the pathophysiological mechanisms behind age-dependent incomplete penetrance in *MYBPC3* carriers. For example, mice lacking the regulatory subunit of protein kinase A (PKA) complex have extended lifespans and are resistant to cardiac dysfunction, which explains its significance in aging and cardiac function. Previous literature identifies PKA as a target for aging and the aging heart and proposes it as a therapeutic target to rescue aging phenotypes^[220,221]. Similarly, the deregulation of c-Jun NH₂-Terminal kinase (JNK), CaMKII^[222], and Protein Kinase B (PKB)^[223,224] in animal models of aging has been ascribed to the reduced immune and neurological functions in the elderly, and these kinases are considered biological markers of aging.

To understand the pathophysiological mechanism in age-dependent pleiotropy, cMyBP-C phosphorylation mechanisms have been investigated extensively. A total phosphorylation quantitation of myofilament proteins in the hearts of naturally aging mice determined that the phosphorylation status of cMyBP-C increased with age in both sexes^[225]. Additionally, this study determined that cMyBP-C phosphorylated at Ser²⁹⁵ and Ser³¹⁵ positions was increasingly expressed in neonatal hearts compared to adult hearts. These findings suggest a protective role in the neonatal heart against hypoxia and acidosis^[226] and establish a possible correlation between aging, heart disease, and the phosphorylation status of cMyBP-C. To further understand, wild-type, phospho-mimetic (mutated at Ser²⁷³, Ser²⁸², Ser³⁰² sites to aspartic acids), and phospho-ablated cMyBP-C (mutated at Ser²⁷³, Ser²⁸², Ser³⁰² sites to alanine), transgenic mice were maintained for 18-20 months^[227], mimicking the aging of human subjects (60-70 years). Among these, phospho-mimetic cMyBP-C mice had better survival and better preservation of systolic and diastolic functions, as well as constant wall thickness, compared to wild-type and phospho-ablated cMyBP-C mice. This evidence suggests that phosphorylation of these residues is beneficial in protecting age-related cardiac dysfunction^[227]. In the context of *MYBPC3* variants, genotype-positive HCM patients with R1073W, E542Q, D770N, E461X, L527fs/3, Q791 fs/40, and V1063 fs/63 mutations exhibited diminished phosphorylation at their myosin regulatory light chain compared to noncarriers^[105,228]. While the presence of *MYBPC3* mutations appears to alter phosphorylation levels, more detailed studies are needed to validate their modulations, along with the pathological consequences in late-onset HCM.

Protein quality control and aging

In age-associated severity of HCM, cardiomyocytes undergo a variety of molecular changes, among which UPS could play a major role. To understand the role of UPS in late-onset HCM, age-associated UPS dysfunction needs to be addressed. Dysfunction in protein synthesis and degradation alters the steady state of the macromolecular degradation system and imparts enhanced ER stress and inflammatory signals^[229]. The involvement of UPS is well recognized in senescence and aging events, viz., NF κB signaling, AMPK-activated protein kinase activity, cyclin-dependent kinase and inhibitors, and FOXO transcription-mediated protein processing^[230]. Aging-dependent biological functions, such as immune and inflammatory response, cellular metabolism, autophagy, and cellular proliferation, also require extensive protein processing, in part from UPS.

Among the many pathways defined for cardiac diseases, macromolecular dynamics have been considered closely related to cardiac aging. Among the protein and nucleic acids dynamics, reduced protein degradation pathways are the major pathway discussed in many age-related phenotypes, including cardiac aging and HCM^[170,230-233]. Using the longest-living noncolonial animal, *Arctica islandica*, it was concluded that maintaining protein homeostasis is essential to the preservation of cardiac function and that low-grade chronic inflammation in the cardiovascular system is a hallmark of the aging process^[233,234]. Similar results were observed in Fisher 344 rat hearts, where proteasome activity was shown to decrease with age, thus resulting in the loss of proteasome function. Consequently, dysregulation of protein homeostasis results in ubiquitinated protein accumulation in the aged cardiomyocytes and heart^[62,66]. Abnormal inclusion bodies are characteristic of neurodegenerative disease, where impaired protein homeostasis plays a major role. Similarly, cardiac amyloids were also detected in patients with amyloidosis^[235]. The development of HF was also associated with inappropriate protein metabolism. These events led to the inability of cardiomyocytes to elicit an appropriate response to age-related stress and associated cardiovascular complications.

As cMyBP-C variants are subjected to UPS-mediated protein degradation, it is reasonable to presume that an age-dependent decline in UPS might result in enhanced mutant protein production at later ages. Indeed, the same phenomenon has been defined experimentally where impairment in the UPS system was observed in *MYBPC3* mutant genotypes^[170], as well as UPS saturation or impairment in older *MYBPC3* knock-in mice^[236]. Although HSC70, a chaperone-mediated autophagy-related protein, was recognized as the abundant protein interactor of *MYBPC3*, mutations in *MYBPC3* did not affect HSC70 localization, nor did it induce a protein folding stress response or ubiquitin proteasome dysfunction^[175]. However, dysfunction in chaperone-mediated autophagy was well documented in aging^[176]; notably, HSC70 and BAG3 have been defined as having a potential role in aging^[237-240]. As discussed above, alterations in the proteasome and chaperone-mediated autophagy are involved in aging, senescence, and regulation of lifespan, and their decline in function has been attributed to advancing age. These findings indicate that the enhanced existence of mutant protein at older ages results in poison polypeptide and dominant negative mechanisms, increasing the severity of HCM phenotypes in older populations.

CALCIUM SENSITIVITY IN HCM AND AGING

Cardiomyocyte functions depend on the force generated by myofilaments. Myocyte calcium concentrations are essential for actin-myosin interactions in the myofilaments. Intracellular calcium reserves in the sarcoplasmic reticulum (SR) and sarcomeric units are released upon electrical stimuli and are sequestered back to restore the calcium in sarcoplasmic reticulum (SR) through Ca^{2+} -ATPase^[241]. The excitation-contraction coupling process involves cell membrane excitation and subsequent contraction by an action potential. The action potential resulting in cell membrane depolarization is followed by a rise in intracellular calcium levels. The calcium ions released from the endoplasmic reticulum attach themselves to the calcium-binding component of troponin, known as troponin-C (TnC), whose interaction counteracts the inhibitory effects of troponin, leading to the initiation of muscle contraction. Tropomyosin can now move along the actin surface and thus activate contraction^[109]. However, any change in calcium concentrations results in calcium mishandling, leading to perturbed force generation and pathological conditions of cardiac diseases^[242-244]. The role of calcium in cardiomyocyte function, calcium signaling pathways, and many associated genes and proteins, along with their significance in cardiac dysfunction, has been reviewed in detail elsewhere^[245].

Calcium sensitivity in *MYBPC3* carriers

Abnormal calcium (Ca^{2+}) homeostasis is considered one of the molecular mechanisms underlying HCM. Current pharmacological treatments for HCM often include calcium channel blockers, which reduce the

availability of Ca²⁺ and inhibit contractions. In HCM, an increase in calcium sensitivity is recognized as a significant hallmark event. A detailed analysis of either mutation in *MYBPC3* (c.1358dupC, c.1960C>T, and c.2308G>A) alone or along with mutations in other genes, such as Filamin C (FLNC) (c.2234A>G), Lysosomal Associated Membrane Protein-2 (LAMP2; c.1988G>A) or MYH7 (c.1293C>T), revealed that compound mutations exhibited reduced contractile force (F_{max}) with HF compared to single mutations in *MYBPC3*. However, calcium sensitivity was most pronounced in compound mutations with *MYBPC3* mutations^[246]. Further analysis of cardiac tissue samples from individuals carrying 15 different *MYBPC3* mutations associated with HCM showed elevated protein levels of CaMKIIδ, along with increased phosphorylation of pT17-phospholamban, a target of CaMKIIδ. However, *MYBPC3* mutant-mediated HCM was not rescued by crossing the mice with phospholamban knockout mice^[247]. The levels of histone deacetylase (HDAC4) were elevated in the HCM, suggesting a possible epigenetic process activated by CamKII^[248]. These findings provide insights into the effects of cMyBP-C mutations and their association with heightened calcium sensitivity in myocytes of patients with gene variants. Similar results have been observed in studies involving the removal or deficiency of cMyBP-C in myocardial samples, further supporting the link between cMyBP-C mutants and increased calcium sensitivity, which has been extensively reviewed elsewhere^[249].

Calcium homeostasis in aging

Calcium homeostasis is critical for healthy heart function, and its disturbance causes various cardiac complications, including the HCM phenotype^[105,250-252]. Furthermore, higher calcium sensitivity, as observed in *MYBPC3* carriers^[105,250], is requisite to define its role in late-onset HCM in these individuals^[76]. Based on earlier publications, a decrease in calcium transient amplitude has been recognized as a well-known phenomenon associated with age^[225]. As age advances, evidence suggests an increase in diastolic and systolic dysfunction. The role of age in cardiomyocytes with respect to calcium homeostasis and contractile functions has been reviewed in detail by Feridooni *et al.*^[253]. Increased sensitivity to calcium and incidence of diastolic dysfunction are early indicators of *MYBPC3* mutation-mediated HCM^[250], which is also a major phenomenon associated with advanced age. Alterations in calcium sensitivity have been established in *MYBPC3* mutant carriers, but evidence also suggests that calcium handling is not likely to be deregulated in *MYBPC3* mutations where alterations in contractile kinetics are independent of calcium flux^[169]. Consistently, the lack of correlation between calcium and mutant background has been proven using 3D cardiac tissues generated from hiPSC of *MYBPC3* carriers^[254] and phosphorylation site mutant transgenic mice^[255]. With further research to ascertain the significance of calcium signaling in *MYBPC3* carriers, age-dependent calcium dynamics might be confirmed as a potential cause of HCM penetrance in the elderly.

CURRENT TREATMENT OPTIONS

Current research is focused on the correction of sarcomere deficit and rescue from HCM phenotypes. Strategies include gene replacement or gene silencing by anti-sense oligonucleotides or CRISPR-based gene modification, which is in the preclinical stage^[256-258]. CRISPR Cas-based gene modification is in the proof-of-concept stage for *MYBPC3* mutations^[259]. At the cellular energetics level, optimizing cardiac metabolism and mitochondrial function can be targeted as a potential therapeutic intervention. This strategy includes small molecules targeting different sarcomere functions, such as calcium sensitivity, cross-bridge kinetics, tension cost of myofilament, ATP utilization, and super-relaxed state. MYK-461 (mavacamten, myosin inhibitor) has completed its preclinical and clinical trials and is available in the clinic for HCM treatment^[260-262]. Since PTC mutations are the primary activators of the NMD pathway, several other cellular dysfunctions and associated disorders are also affected by cellular NMD, such as senescence and aging. Detailed profiling of FDA-approved drugs for their impact on NMD efficiency identified significant NMD targets and NMD modulators. For example, the anticancer drug homoharringtonine (HHT or omacetaxine mepesuccinate) increased NMD substrates in multiple cell types with concomitant increase in NMD processing^[263].

Antagomirs, also known as anti-miRs, for miR-132 as a treatment for HF^[264,265] and anti-miR-92 for the treatment of cardiovascular disease and wound healing^[266] have been developed, and both have entered preclinical and clinical trials. Considering this, the validation of miRNAs that regulate both wild-type and variant forms of *MYBPC3* holds promise as a potential therapeutic approach for HCM in individuals carrying *MYBPC3* mutations. For example, Tenaya Therapeutics is conducting clinical trials involving the overexpression of cMyBP-C in patients with HCM (TN-201, Clinical Trial ID: NCT05836259). Apart from these, focusing on age-related pathways could also reduce the effects of age-dependent increase in HCM severity. Interventions, such as caloric restriction and intermittent fasting, expression of cardiomyocyte-specific dnPI3K and transgenic overexpression of Parkin, low to moderate SIRT1 in transgenic mouse hearts^[267], protein kinase B β (Akt2) knockout, spermidine, Rapamycin, Resveratrol, and SRT1720 treatment, have resulted in modulating SIRT1, mTOR, AMPK and FOXO pathways and delayed cardiac aging, thus preserving cardiac function in aged animal models^[268]. A few other common drug options include Metformin, ACE inhibitors, Aspirin, Statins, β -blockers, AT1 blockers, Omecamtiv mecarbil, Berberine, polyunsaturated fatty acids (PUFAs), NFE2 like bZIP transcription factor 2 (Nrf2) activators and Mito-targeted antioxidants^[269]. With a growing number of drugs and treatment options, demethylation agent 5-azacytidine, displaying antifibrosis and anti-hypertrophic properties^[270], will be a promising strategy to prevent hypermethylation of CpG islands of *MYBPC3*. Thus, along with the available advanced diagnostic and therapeutic interventions, nondrug-based (diet and physical exercise) or drug-based modulations of gene regulation and signaling pathways related to hypertrophic phenotype and aging could afford elderly *MYBPC3* carriers a better chance of early diagnosis and prevention of HCM severity.

CONCLUSIONS

The age-dependent incomplete penetrance of *MYBPC3* mutations is characterized by heterogeneous clinical presentation mainly affecting elderly carriers, whereas younger individuals experience SCD more often. *MYBPC3* mutant mRNA and protein are processed by NMD and UPS systems, causing haploinsufficiency or deficiency and a deficit in sarcomere function. This disarrayed sarcomere structure and function trigger a stress-sensing mechanism that causes cellular perturbations, such as changes in epigenetic factors and altered signaling pathways, leading to the initiation of the hallmark events of cardiac hypertrophy. To preserve functional myofibrillar components and cardiac function, it is essential to identify modulators of *MYBPC3* mutant gene and protein expression like epigenetic factors, transcription factors, and proteins associated with AS, NMD, and UPS pathways. Dysregulation of any of these checkpoints would have a significant impact on the onset of HCM disease phenotype. Age-associated alterations of *MYBPC3* modulators also play a role in triggering increased severity of HCM in the elderly.

Some potential treatment options have emerged at various levels of preclinical and clinical trials. Needless to say, strategies aimed at ameliorating the severity of HCM in the elderly require additional focus on identifying potential biomarkers, therapeutic targets, and novel drugs. High-throughput RNA sequencing, small RNA sequencing, and ChIP sequencing in elderly human HCM tissue, as well as animal models of human aging, will be valuable tools in the selection of key target molecules, genes, and pathways for therapeutic intervention^[271].

DECLARATIONS

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The graphical abstract was created with BioRender.com.

Authors' contributions

Conceptualization, investigation and project administration: Ananthamohan K, Sadayappan S

Visualization and writing (original draft): Ananthamohan K

Funding acquisition: Sadayappan S, Stelzer JE

Supervision: Sadayappan S

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Conflict of interest

Sadayappan S provides consulting and collaborative research studies to the Leducq Foundation (CURE-PLAN), Red Saree Inc., Greater Cincinnati Tamil Sangam, Affinia Therapeutics Inc., and Cosmogene Skincare Private Limited, but such work is unrelated to the content of this article. Dr. Ananthamohan K and Dr. Stelzer JE have no conflicts of interest to disclose.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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