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REVIEW

Genetic, clinical, molecular, and pathogenic aspects of the South Asian–specific polymorphic *MYBPC3*$^{\Delta 25bp}$ variant

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Abstract

Hypertrophic cardiomyopathy (HCM) is a cardiac genetic disease characterized by ventricular enlargement, diastolic dysfunction, and increased risk for sudden cardiac death. Sarcomeric genetic defects are the predominant known cause of HCM. In particular, mutations in the myosin-binding protein C gene (*MYBPC3*) are associated with ~40% of all HCM cases in which a genetic basis has been established. A decade ago, our group reported a 25–base pair deletion in intron 32 of *MYBPC3* (*MYBPC3*$^{\Delta 25bp}$) that is uniquely prevalent in South Asians and is associated with autosomal dominant cardiomyopathy. Although our studies suggest that this deletion results in left ventricular dysfunction, cardiomyopathies, and heart failure, the precise mechanism by which this variant predisposes to heart disease remains unclear. Increasingly appreciated, however, is the contribution of secondary risk factors, additional mutations, and lifestyle choices in augmenting or modifying the HCM phenotype in *MYBPC3*$^{\Delta 25bp}$ carriers. Therefore, the goal of this review article is to summarize the current research dedicated to understanding the molecular pathophysiology of HCM in South Asians with the *MYBPC3*$^{\Delta 25bp}$ variant. An emphasis is to review the latest techniques currently applied to explore the *MYBPC3*$^{\Delta 25bp}$ pathogenesis and to provide a foundation for developing new diagnostic strategies and advances in therapeutics.

Keywords Hypertrophic cardiomyopathy · Heart failure · MYBPC3 · South Asian

Introduction

Hypertrophic cardiomyopathy (HCM), one of the leading forms of cardiovascular disease (CVD) in the world, is a genetic disorder that affects ~ 20 million people globally, including 750,000 Americans. HCM is characterized by ventricular expansion, resulting in diastolic dysfunction and an increased risk of sudden death (Gersh et al. 2011). Sarcomeric genetic defects are the predominant cause of HCM (Bonne et al. 1995; Carrier et al. 1998; Gersh et al. 2011; Harris et al. 2011; Spirito et al. 1997; Watkins et al. 1995; Watkins et al. 1993). Several reports have established a direct link between sarcomeric mutations and cardiomyopathies, including HCM and dilated cardiomyopathy (DCM) (Hitomi et al. 2010; Niimura et al. 2002; Tabish et al. 2019; Tanjore et al. 2008; Van Driest et al. 2005).

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HCM is termed a disease of the sarcomere as mutations in sarcomeric genes, such as *MYH7*, *MYBPC3*, *MYL3*, *TPM1*, *TNNI3*, and *TNNT2*, are the principal causes of pathology (Geisterfer-Lowrance et al. 1990; Seidman and Seidman 2011; Thierfelder et al. 1994). However, 5–10% of HCM cases are caused by mutations in genes involving metabolic pathways (MacRae et al. 1995; Murphy et al. 2005). Importantly, mutations in both *MYH7* and *MYBPC3* genes contribute to more than 80% of HCM cases (Viswanathan et al. 2017), of which *MYBPC3* is the leading gene known to cause HCM and DCM (Bonne et al. 1995; Previs et al. 2012; Viswanathan et al. 2017). The *MYBPC3* gene encodes a thick-filament cardiac muscle protein, a cardiac paralog of myosin-binding protein C (cMyBP-C), which is involved in regulating myosin function, rate of force generation, and cardiac contractility. Also, *MYBPC3* mutations present a high risk for heart failure (HF) (Adalsteinsdottir et al. 2014; Barefield et al. 2014; Dhandapany et al. 2009; Helms et al. 2014; Michels et al. 2009; Page et al. 2012). Previously, our group discovered a polymorphic variant in *MYBPC3*, a 25–base pair deletion (***MYBPC3***$^{\Delta 25bp}$) at intron 32 (Fig. 1). One of the molecular consequences of *MYBPC3*$^{\Delta 25bp}$ is the replacement of 65 wild-type amino acids by 58 novel amino acids in the carboxyl region of cMyBP-C (Dhandapany et al. 2009; Kuster et al. 2015; Kuster and Sadayappan 2014; Waldmuller et al. 2003). The variant is associated with HCM, DCM, and HF (Dhandapany et al. 2009; Simonson et al. 2010; Srivastava et al. 2011).

Discovery of the *MYBPC3*$^{\Delta 25bp}$ mutation and its association with cardiomyopathies was first noted at the International Society for Heart Research in Winnipeg, Canada, on July 6–11, 2001 (Sakthivel et al. 2001). In our first study, *MYBPC3*$^{\Delta 25bp}$ was discovered in two unrelated Indian families in association with the development of HCM (Waldmuller et al. 2003). Since *MYBPC3*$^{\Delta 25bp}$ is familial with late-onset in aging and a mild form of HCM in carriers, the existence of unequivocal disease penetrance has been concluded. The study further determined that *MYBPC3*$^{\Delta 25bp}$ could be a canonical monogenic risk with low expressivity and penetrance. However, whole-genome DNA sequencing was not available at that time, and it was unclear whether the deletion was, in fact, both necessary and sufficient to cause HCM, or, indeed, whether other known or unidentified genetic or risk factors were at play (Waldmuller et al. 2003).

Following these initial investigations, a series of population-based surveys revealed a striking prevalence in the South Asian (SA) population (Table 1), estimated to be present in 6% and translating to ~ 100 million people worldwide (Dhandapany et al. 2009; Viswanathan et al. 2018) (Fig. 2). For example, Dhandapany and colleagues suggested *MYBPC3*$^{\Delta 25bp}$ prevalence around 2–6% in Southeast Asians. They further suggested that the higher incidence of cardiomyopathies in SAs, compared with other ethnicities, could be, at least partly, attributed to this polymorphism (Dhandapany et al. 2009; Dodani 2008; Gupta and Brister 2006; Jones et al. 2014; Omariba 2015; Uppaluri 2002). More recently, Kumar et al. (2016) studied the frequency of sarcomeric gene polymorphisms with left ventricular dysfunction (LVD) in patients with coronary artery disease (CAD). Contrary to other sarcomeric gene mutations, such as titin, troponin T type 2, and myospryn, they showed the *MYBPC3*$^{\Delta 25bp}$ polymorphism to be associated with an elevated risk of left ventricular pathologic remodeling and cardiac dysfunction post-myocardial infarction (Kumar et al. 2016; Srivastava et al. 2011). The association of *MYBPC3* mutations with HCM and sudden death, the high prevalence of this mutation in the SA community, and preclinical studies revealing pathologic consequences of the *MYBPC3* mutation all support ongoing effort to understand the molecular mechanisms underlying *MYBPC3*

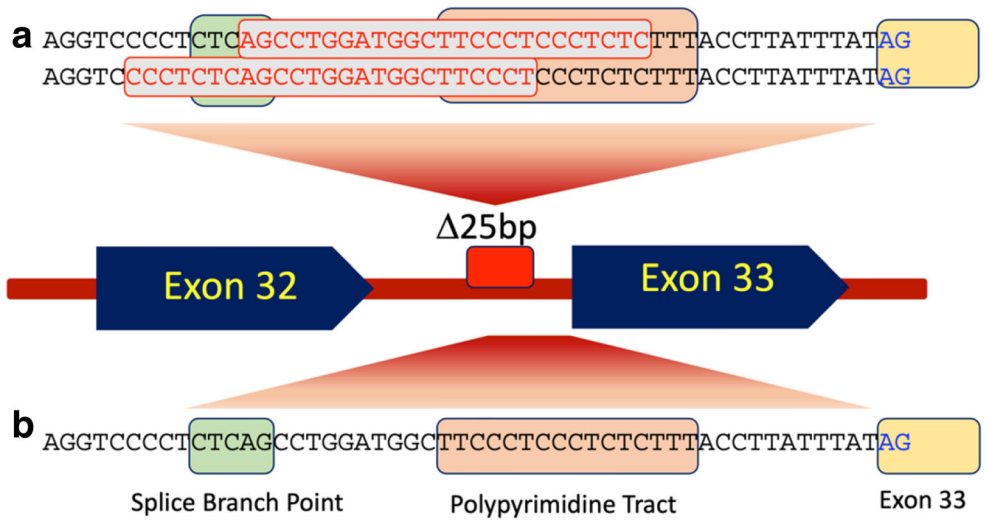

Fig. 1 Genotype of *MYBPC3*$^{\Delta 25bp}$ in intron 32 of the *MYBPC3* gene. (**a**) Two options of 25-bp deletion are indicated with the same outcome. (**b**) The location of the splice branch point and polypyrimidine track at the junction of intron 32/exon 33 splicing (modified from Sadayappan et al. 2020)

Table 1 The global distribution and frequency of subjects found to be carrying *MYBPC3*$^{\Delta 25bp}$ according to the country of residence

Countries/ region	Total subjects analyzed	*MYBPC3*$^{\Delta 25bp}$ carriers	Frequency (%)	Reference
Southern India	229	16	6.98	Waldmuller et al. (2003)
India	6159	291	4.72	Dhandapany et al. (2009)
Pakistan	770	32	4.15	Dhandapany et al. (2009)
Sri Lanka	21	2	9.52	Dhandapany et al. (2009)
Malaysia	272	4	1.47	Dhandapany et al. (2009)
Indonesia	61	1	1.63	Dhandapany et al. (2009)
Southeast Asia	220	5	2.27	Srivastava et al. (2011)
Europe	110,751	170	0.15	Chowdry et al. (2012) (23&Me)*
USA	2401	144	5.99	Viswanathan et al. (2018)**

**Denotes the screening of South Asians residing in the USA; South Asians include those from Afghanistan, Bangladesh, Bhutan, India, Maldives, Myanmar, Nepal, Pakistan, and Sri Lanka

*Denotes the screening of subjects of all ethnicities in Europe

mutation–mediated HCM. The objective of our review is to detail the association of *MYBPC3*$^{\Delta 25bp}$ and HCM/HF in SAs, describe the pathophysiology of HCM/HF in the setting of *MYBPC3* mutations, and explore newly proposed diagnostic and therapeutic strategies. Overall, we aim to provide a constructive answer to the frequently posed question by SAs: "I recently found out through genetic testing (23andme) that I have one copy of the MYBPC3 mutation [25bp deletion]… should I be taking any precautions?"

Increased risk of cardiovascular disease in South Asians

At 1.8 billion people, SAs comprise approximately 20% of the world's population. An estimated 3.5 million SAs live in the USA, constituting approximately 1% of the American population (Tang et al. 2012). Despite being only 20% of the world's population, SAs represent about 60% of CVD cases worldwide (Kraker et al. 2016), suggesting a unique predisposition worthy of increased investigation. The World Health Organization (WHO) reports that CVD takes the lives of 17.9 million people every year, accounting for 31% of all deaths globally. Strikingly, out of those 17.9 million people, 13.6%, or 2.43 million, are of SA origin (Finegold et al. 2013; Volgman et al. 2018), including Sri Lanka, Nepal, Bangladesh, Bhutan, India, and Pakistan. Interestingly, the SA population has a higher risk of CVD than any other ethnic group (Gupta and Brister 2006). While researchers have advanced many causes to explain the high incidence of CVD among SAs, the literature has been circumspect and inconclusive, and, to date, no reports have pinpointed with precision

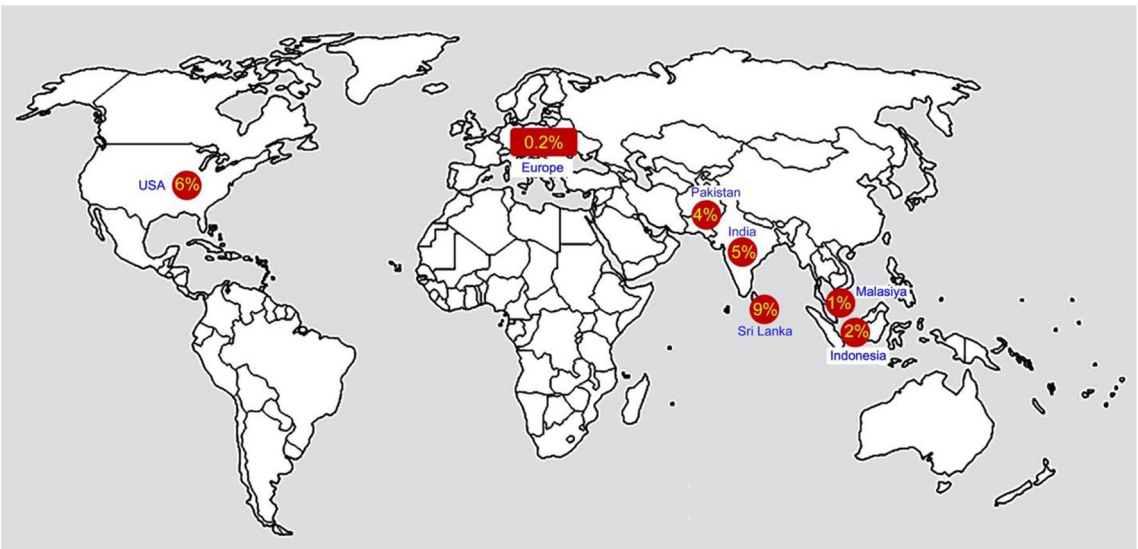

Fig. 2 Prevalence of *MYBPC3*$^{\Delta 25bp}$ worldwide. The frequency of *MYBPC3*$^{\Delta 25bp}$ distribution in various countries is shown as a percentage. Details are provided in Table 1 with the total number of samples screened by the country concerning the existing literature

the etiology of increased risk for CVD among SAs. MetS (metabolic syndrome) is a group of disease conditions of metabolism (Pan et al. 2008) associated with an increased risk for heart disease (Ram and Farmer 2012). The conditions associated with this designation are all risk factors for CVD (Kaur 2014), and they include hypertension, elevated triglyceride/HDL ratio, hyperinsulinemia, insulin resistance, abdominal adiposity, and dyslipidemia (Huang 2009; Pan et al. 2008; Ram and Farmer 2012). Around 20–25% of SAs are known to currently have MetS (Eapen et al. 2009), indicating that MetS could play an important pathogenic role in the susceptibility of SAs to CVD. A key component of MetS is insulin resistance (Eapen et al. 2009). When compared with Caucasians, SAs have notably been found to be at more risk for insulin resistance (Simmons et al. 1991), which is a key component of MetS (Eapen et al. 2009). Similarly, the high frequency of *MYBPC3*$^{\Delta 25bp}$ mutation among SAs (~6%) (Dhandapany et al. 2009; Simonson et al. 2010) (Viswanathan et al. 2018) is presumed to be a significant contributor to the high incidence of CVD, along with these secondary risk factors (Srivastava et al. 2011). The challenge is to determine if and how comorbidities, such as diabetes and hypertension, contribute to the ultimate phenotype and natural history of *MYBPC3*$^{\Delta 25bp}$ in a specific individual.

cMyBP-C is a regulator of contractility

MYBPC3 encodes cMyBP-C, a key structural protein of the heart muscle that interacts with myosin (Flavigny et al. 2003; Flavigny et al. 1999; Gruen and Gautel 1999; Shaffer et al. 2009), titin (Freiburg and Gautel 1996), and actin (Shaffer et al. 2009; Squire et al. 2003) to support sarcomeric integrity (Fig. 3). cMyBP-C was first discovered in the 1970s as a contaminant in a myosin preparation (Bennett et al. 1986; Craig and Offer 1976; Starr and Offer 1971). The gene encoding the cardiac isoform was later identified and characterized (Carrier et al. 1997; Carrier et al. 1993; Freiburg and Gautel 1996; Oakley et al. 2004). *MYBPC3* is a single-copy, 24-kb gene, consisting of 35 codons encoding a 1274–amino acid and 140-kDa protein. Mutations involving the *MYBPC3* gene cause significant cardiac disease at all ages (Barefield and Sadayappan 2010; Chung et al. 2003; Maron 1996; Van Driest et al. 2004). Experimental evidence, mainly resulting from in vitro protein-protein interaction studies, suggests that cMyBP-C may serve two functions: one as a molecular "ruler," coordinating the spacing between thick and thin filaments, and one as a regulator of actomyosin interaction by association with the myosin II neck region (S2) and F-actin. cMyBP-C encodes multiple repeats of fibronectin type III–like and immunoglobulin (Ig)-like domains but differs from skeletal isoforms in that it contains an N-terminal C0 domain, a proline/alanine-rich linker between C0 and C1 and an ~100 amino acid segment between C1 and C2 Ig domains (the M-domain). The M-domain contains multiple serine residues that are conserved between mice and humans and are reversibly phosphorylated in response to systolic pressure and adrenergic stimulation (Kulikovskaya et al. 2003b), thereby modulating myofilament affinity (Kooij et al. 2013; Mun et al. 2011). Genetic profiles have been used to study how changes in cMyBP-C regulation at the molecular and cellular levels affect the sarcomere structure. However, the correlation between sarcomeric defects and organ-scale defects of myocyte organization remains an open area for investigation. The phosphorylation of cMyBP-C plays an important role in the regulation of cardiac mechanics (Rosas et al. 2015; Rosas et al. 2019; Tong et al. 2008; Tong et al. 2015). Sadayappan and colleagues have demonstrated that total cMyBP-C phosphorylation affects cardiac contractility, sarcomere organization, and the response to ischemia-reperfusion (I/R) injury (Sadayappan et al. 2009; Sadayappan et al. 2005; Sadayappan et al. 2006). In vitro experiments suggest that genetic mutations in cMyBP-C could alter interactions within the sarcomere, such as the α-tropomyosin and light meromyosin region of myosin (James and Robbins 2011; Okagaki et al. 1993), the S2 region of myosin (Gruen and Gautel 1999), F-actin (Colson et al. 2012; Kensler et al. 2011; Shaffer et al. 2009), and titin (Al-Khayat et al. 2013). Moreover, the M-domain, containing serine residues differentially phosphorylated by cAMP-dependent protein kinase (Gautel et al. 1995; Hartzell and Titus 1982) and endogenous calcium/calmodulin-dependent kinase (Hartzell and Glass 1984), may modulate contractility (Stelzer et al. 2006) through tethering of the S2 region of myosin (Weisberg and Winegrad 1996) and by modulation of actin-binding at the N-terminus (Kulikovskaya et al. 2003a; Razumova et al. 2006). Nuclear magnetic resonance studies suggest that the first 140 amino acids of the M-domain contain regulatory phosphoserines flanked by N-terminal charged residues. Phosphorylation of the M-domain leads to a transient helical structure, whereas a more stable trihelical structure comprises the C-terminal portion of the M-domain (Howarth et al. 2012).

As revealed in previously published in vitro results (Kulikovskaya et al. 2003a), the N-terminal region relative to the M-domain is required for the interaction between M-domain and F-actin. Together, these data support the hypothesis that mutations in the gene encoding cMyBP-C affect the signaling platform regulating actin and myosin and cause an altered contraction in the heart (Charron et al. 1998; Kooij et al. 2013; Niimura et al. 1998). In addition, we previously demonstrated in rats with induced myocardial infarction that the normal left ventricular architectural pattern is replaced by a largely disordered region, upon which a mesh-like network of orthogonally oriented myofibers is superimposed, extending from within the infarct to the septal and basal border zones. Addressing the underlying mechanism of this architectural

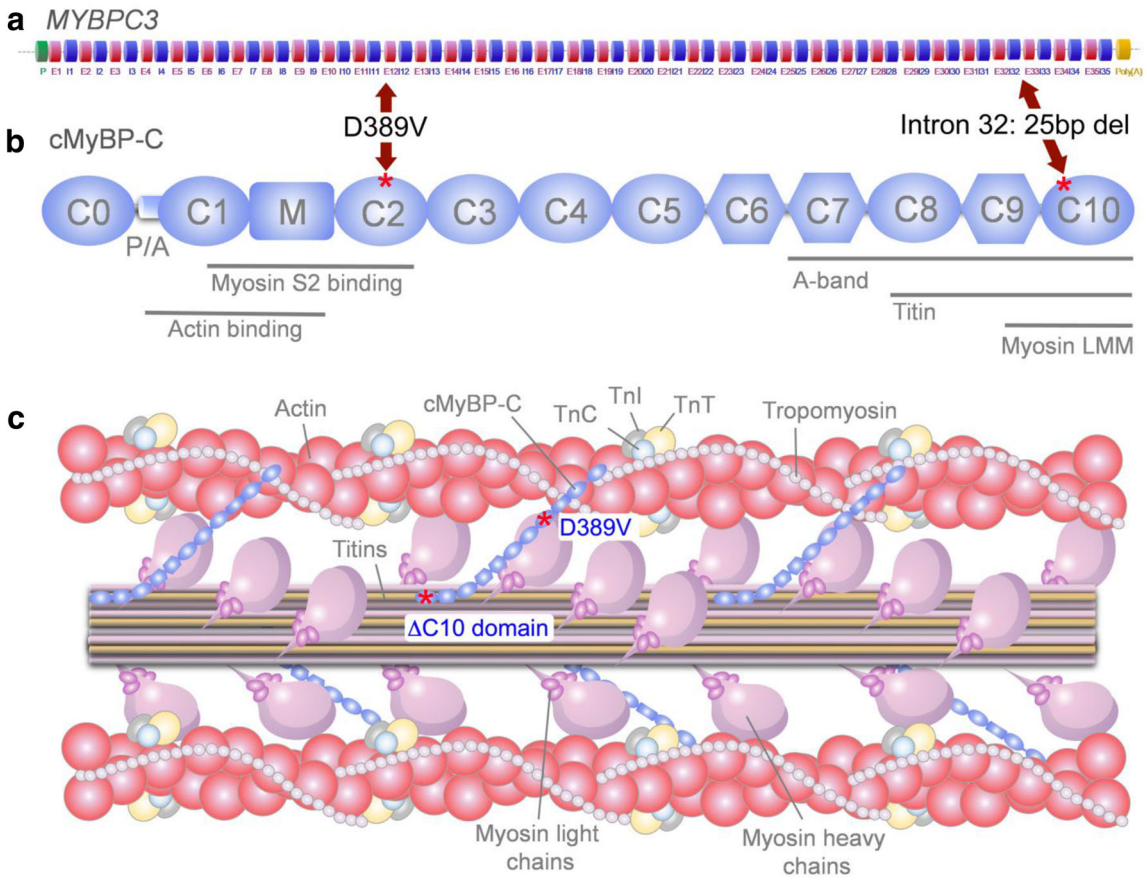

Fig. 3 cMyBP-C: structure, localization, and function illustrating the location of both *MYBPC3*$^{\Delta 25bp}$ and *MYBPC3*^{D389V} variants. (**a**) *MYBPC3* gene comprising 35 exons and 35 introns. (**b**) cMyBP-C codes for 1273 amino acids of cMyBP-C protein containing several domains. (**c**) cMyBP-C is located on 7–9 stripes of 43 nm spacing in each half of the A-band (cross-bridge bearing zone, C-region) of the sarcomere exclusively in cardiac myocytes. Both *MYBPC3*$^{\Delta 25bp}$ and *MYBPC3*^{D389V} variants are indicated in the *MYBPC3* gene and cMyBP-C protein. cMyBP-C is an important structural component of the sarcomere which plays a regulatory role in cardiac muscle function via interacting with actin, myosin, and titin. cMyBP-C, cardiac myosin-binding protein C; *MYBPC3*, cardiac myosin-binding protein C3 gene

disorder, the Sadayappan laboratory demonstrated that an N-terminal 40-kDa fragment of cMyBP-C during I-R injury is generated from calpain-dependent cleavage at 272-TSLAGAGRRTS, near a conserved protein kinase A phosphorylation motif, containing the cardiac-specific C0 domain (99 amino acids), C1 domain, and the first 17 residues of the M-domain (C0-C1f) (Govindan et al. 2012; Sadayappan and de Tombe 2012). Since C0-C1f lacks the phosphorylation sites for dynamic interplay involving actin and myosin S2, the presence of the cleaved product during I/R may be responsible for impaired sarcomere structure and function, leading to altered macroscopic myoarchitecture and impaired mechanics (Barefield et al. 2019; Hoffman et al. 2018; Taylor et al. 2016). Moreover, it is well known that I/R promotes a reduction in cMyBP-C phosphorylation, notably via a loss in triphosphorylated species (Sadayappan et al. 2006), and that cMyBP-C phosphorylation confers resistance to proteolysis (Barefield et al. 2019) while protecting against I/R injury (Sanada et al. 2004). Extending these concepts regarding the central role of cMyBP-C in affecting sarcomere structure, it is reasonable to postulate that the tissue architectural response to I/R would similarly be regulated by cMyBP-C and its degree of phosphorylation. Therefore, it is obvious that mutations in genes encoding for cMyBP-C would likely affect sarcomere structure, regulation, and function.

Potential mechanism of cardiomyopathies owing to MYBPC3$^{\Delta 25bp}$

Currently, more than 350 disease-causing *MYBPC3* mutations have been identified, and most are associated with HCM (Carrier et al. 2015; Dhandapany et al. 2009; Morimoto 2008; Stenson et al. 2014). Importantly, several founder mutations in different populations/countries were identified and reported, including Japan (Kubo et al. 2005), Finland (Jaaskelainen et al. 2013), Iceland (Adalsteinsdottir et al. 2014), France (Teirlinck et al. 2012), Spain (Oliva-Sandoval et al. 2010), the USA (Saltzman et al. 2010), the Netherlands (Alders et al. 2003) and *MYBPC3*$^{\Delta 25bp}$ from South Asia

(Dhandapany et al. 2009). It is likely that such an effect existed in SA countries some 40,000 years ago (Dhandapany et al. 2009). Furthermore, the chance of developing HCM among *MYBPC3*$^{\Delta 25bp}$ carriers is increased by 5.3-fold odds ratios among independent subjects with HF (Dhandapany et al. 2009). This calls for research focused on discovering the pathophysiological mechanism of *MYBPC3*$^{\Delta 25bp}$ relative to cardiomyopathies. Interestingly, 70% of total *MYBPC3* mutations are premature stop codons or frameshifts at the carboxyl region of cMyBP-C, leading to truncations lacking key myosin-binding residues. Truncations in domains C7 to C10 of the carboxyl region of cMyBP-C prevent its incorporation into the sarcomere (Barefield and Sadayappan 2010; Harris et al. 2011; Kuster and Sadayappan 2014). Other underlying mechanisms may include (1) "splicing defects," an insufficient amount, or "haploinsufficiency," of *MYBPC3* product (Barefield et al. 2015; Marston et al. 2009); (2) a direct pathogenic effect of a mutant on typical myofilament function, i.e., "poison polypeptide effect" (Kuster et al. 2019); and (3) accumulation of misfolded proteins, i.e., "proteotoxicity" (Kuster et al. 2019) (Fig. 4). The possible mechanisms for *MYBPC3*$^{\Delta 25bp}$ pathogenesis remain uncertain, but evidence suggests the following three possibilities: (i) exon skipping and inclusion of novel amino acids in the coded protein, (ii) haploinsufficiency, or (iii) combination with other mutations on the same allele (Fig. 4). The following sections will discuss each of these mechanisms aiming to understand how *MYBPC3*$^{\Delta 25bp}$ could be pathogenic and cause contractile dysfunction.

Altered splicing and exon 33 skipping owing to MYBPC3$^{\Delta 25bp}$ could be pathogenic

The deletion in intron 32 was potentially expected to cause full skipping of the downstream exon 33, resulting in a reading frameshift, subsequently skipping the stop codon in exon 34. Translation then continued through exon 34 and a part of the 3′-UTR, finally stopping in 3′-UTR (Figs. 3 and 5) (Dhandapany et al. 2009; Waldmuller et al. 2003). In the mouse model, *MYBPC3*$^{\Delta 25bp}$ leads to the replacement of the last C-terminal 65 amino acids with 50 unique amino acids (cMyBP-C$^{\Delta C10mut}$) (Kuster et al. 2015). In this way, *MYBPC3*$^{\Delta 25bp}$ creates a newly altered C10 domain sequence and translates cMyBP-C$^{\Delta C10mut}$ protein lacking exon 33 (Dhandapany et al. 2009; Waldmuller et al. 2003). Reports show that cMyBP-C$^{\Delta C10mut}$ cannot link cMyBP-C to the myosin LMM region, resulting in contractile dysfunction (Hossain et al. 2019; Kuster et al. 2015). Kumar et al. have studied models for cMyBP-C that depict the front and back helical views of cMyBP-C and cMyBP-C$^{\Delta C10mut}$ protein. Interestingly, cMyBP-C$^{\Delta C10mut}$ introduces β-pleated sheets and α-helix that might decrease the binding capacity of cMyBP-C to the myosin LMM region (Kumar et al. 2016). Since carboxyl domains of cMyBP-C directly bind to myosin, titin, and connectin to form normal sarcomere structure, conformational changes caused by cMyBP-C$^{\Delta C10mut}$ will affect these interactions, resulting in severe sarcomere disorganization and leading to structural and functional changes in cardiac muscle (Hossain et al. 2019; Kumar et al. 2016).

In earlier studies, the molecular consequence of *MYBPC3*$^{\Delta 25bp}$ was determined using neonatal cardiomyocytes in vitro and demonstrated that *MYBPC3*$^{\Delta 25bp}$ could skip exon 33 in the transcription (Waldmuller et al. 2003). In a follow-up study, the presence of exon 33 skipping in mRNA was confirmed using a biopsy from an HCM patient with *MYBPC3*$^{\Delta 25bp}$ (Dhandapany et al. 2009). Exon 33 skipping leads to a loss of 62 native cMyBP-C amino acids in the C10 domain (cMyBP-C$^{\Delta C10mut}$). The C10 domain of cMyBP-C directly interacts with the myosin LMM region, which is critical for cMyBP-C anchoring, localization, and stabilization of the cardiac sarcomere. Furthermore, previous studies have established that modification of the C10 domain of cMyBP-C will result in the removal of mutant cMyBP-C since it is not incorporated in the sarcomere (McConnell et al. 1999). In addition, carboxyl domains C7 to C10 of cMyBP-C interact with titin, promoting cMyBP-C integration into the C-zone of the A-band. Kuster et al. (2019) have analyzed the effects of cMyBP-C$^{\Delta C10mut}$ overexpression in isolated cultured cardiomyocytes in vitro (Kuster et al. 2015) and in transgenic mouse hearts in vivo. Results showed that cMyBP-C$^{\Delta C10mut}$ expression is sufficient to cause the HCM phenotype. Altogether, these data suggest that the expression of cMyBP-C$^{\Delta C10mut}$ in cardiomyocytes results in the development of contractile dysfunction and HCM. Alternatively, it could also be hypothesized that the expression of cMyBP-C$^{\Delta C10mut}$ mRNA in cardiomyocytes would result in susceptibility to rapid degradation. This effect was previously shown in a mouse model where exon 31 was mutated such that the mutant RNA does not exist and if it does exist, it does not translate (McConnell et al. 1999). In that specific mouse model, a homozygous mutation of *MYBPC3* in the C-terminus is guaranteed to cause cMyBP-C null, resulting in the HCM phenotype and possible sudden death at an early age (Zahka et al. 2008). On this basis, we propose that *MYBPC3*$^{\Delta 25bp}$ could be pathogenic. However, we still do not know how *MYBPC3*$^{\Delta 25bp}$ promotes HCM. Recent studies suggest that *MYBPC3*$^{\Delta 25bp}$ may not be pathogenic in the context of HCM (Harper et al. 2020; Sadayappan et al. 2020; Viswanathan et al. 2018). However, we need a systematic study to determine whether *MYBPC3*$^{\Delta 25bp}$ is or is not pathogenic (Hossain et al. 2019). One possibility is that the presence of MetS may aggravate the effect of *MYBPC3*$^{\Delta 25bp}$ activation and cause HF (Sadayappan et al. 2020). Future research should consider questions such as (i) the factors that promote *MYBPC3*$^{\Delta 25bp}$ to skip exon 33, (ii) exon 33 skipping under all circumstances or only in the presence of MetS, (iii) any genetic cofactors required to cause exon 33 skipping, and (iv) gender and age association with exon 33 skipping

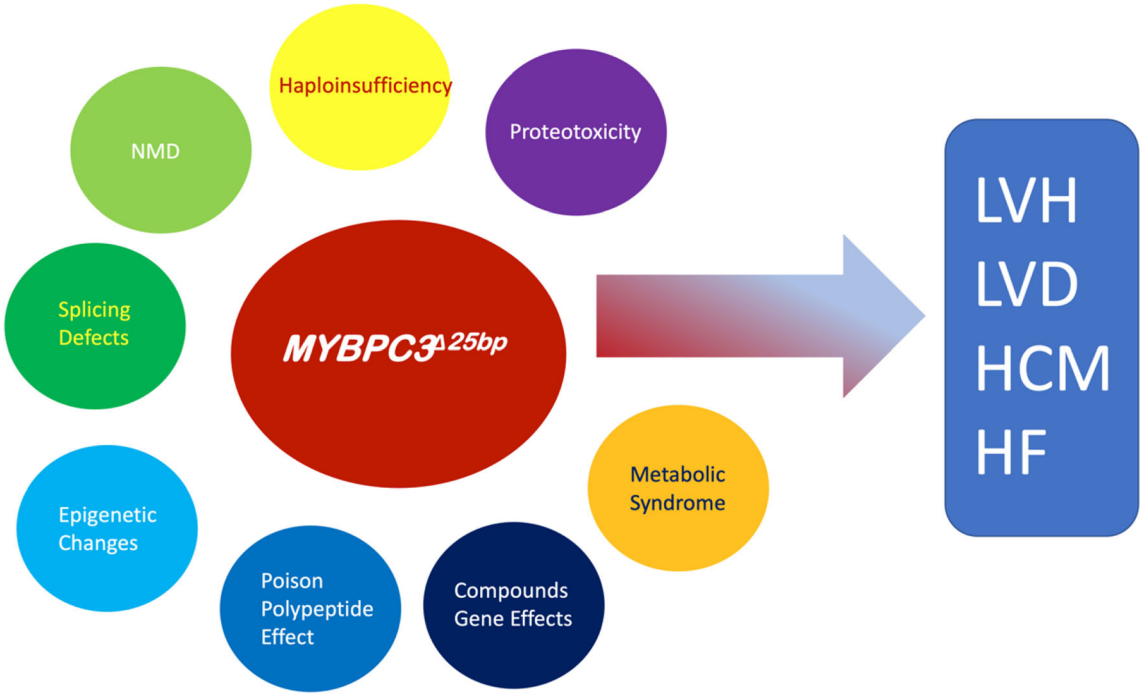

Fig. 4 Possible mechanisms for *MYBPC3*$^{\Delta 25bp}$ pathogenesis. Representation of various possible mechanisms of *MYBPC3*$^{\Delta 25bp}$ in cardiomyocytes with the strong probability of another unidentified phenomenon causing left ventricular hypertrophy (LVH), left ventricular dysfunction (LVD), HCM, and HF. Eight related mechanisms range from abnormal gene transcription to defective translated protein

and the relationship with left ventricular dysfunction (Sadayappan et al. 2020).

Haploinsufficiency

According to the National Institutes of Health, haploinsufficiency is a condition in which "one allele of a gene is inactivated or deleted, and the remaining second functional and normal allele of the gene is not adequate to produce a sufficient amount protein to preserve normal function." In the case of one affected *MYBPC3* allele in a truncated mutation, it appears that the truncated protein is never found in the heart (Rottbauer et al. 1997; van Dijk et al. 2009). Likewise, in patients with the *MYBPC3*$^{\Delta 25bp}$, mutant cMyBP-C was not detected in the biopsies (Dhandapany et al. 2009). It is plausible that haploinsufficiency with the *MYBPC3*$^{\Delta 25bp}$ allele could reduce the total normal cMyBP-C protein level by the failure of the normal allele to fully compensate (Dhandapany et al. 2009). Based on a recent report, the relatively inadequate compensation induces a primary increase in calcium sensitivity which would explain the features observed in HCM patients, including an increased percentage of fractional shortening and ejection fraction, increased calcium transients, presence of myocardial hypertrophy and evidence of relaxation impairments, and increased cardiac remodeling causing myofibrillar disarray and/or life-threatening arrhythmias (Hossain et al. 2019).

The mechanism by which *MYBPC3*$^{\Delta 25bp}$ causes increased myofilament calcium sensitivity is unknown. Nonetheless, mouse models and HCM patient studies suggest that either poison polypeptide or haploinsufficiency of *MYBPC3* would have such an effect (Fraysse et al. 2012; Kuster et al. 2019; Najafi et al. 2016; Sequeira et al. 2013). On the myofilament level, several different mechanisms are proposed to cause HCM, followed by HF and sudden cardiac death (SCD). For example, increased Ca^{2+} handling, inefficient ATP utilization, and accelerated mechanical force in combination could play a role in the development of HCM (Ashrafian et al. 2011; Huke and Knollmann 2010). In addition, mitochondrial energetic abnormalities could also cause increased reactive oxygen species and oxidative stress (Ashrafian et al. 2011; Brouwer et al. 2011), leading to pathologic cardiac remodeling associated with cardiomyopathies like HCM. In support of this mechanism, a recent study shows that cardiac troponin C, the calcium-binding protein in the myofilament, showed increased sensitivity to calcium, meaningless calcium generates more force, which would lead to prolonged muscle tension (Davis et al. 2016). Our group has determined another potential effect of cMyBP-C haploinsufficiency, namely, a reduction in the level of super-relaxed state myosin in which myosin heavy chain is in a low energy state and regulated by cMyBP-C (McNamara et al. 2017; McNamara et al. 2016). This can cause increased energy consumption and, hence, contribute to HCM pathophysiology. In support of this

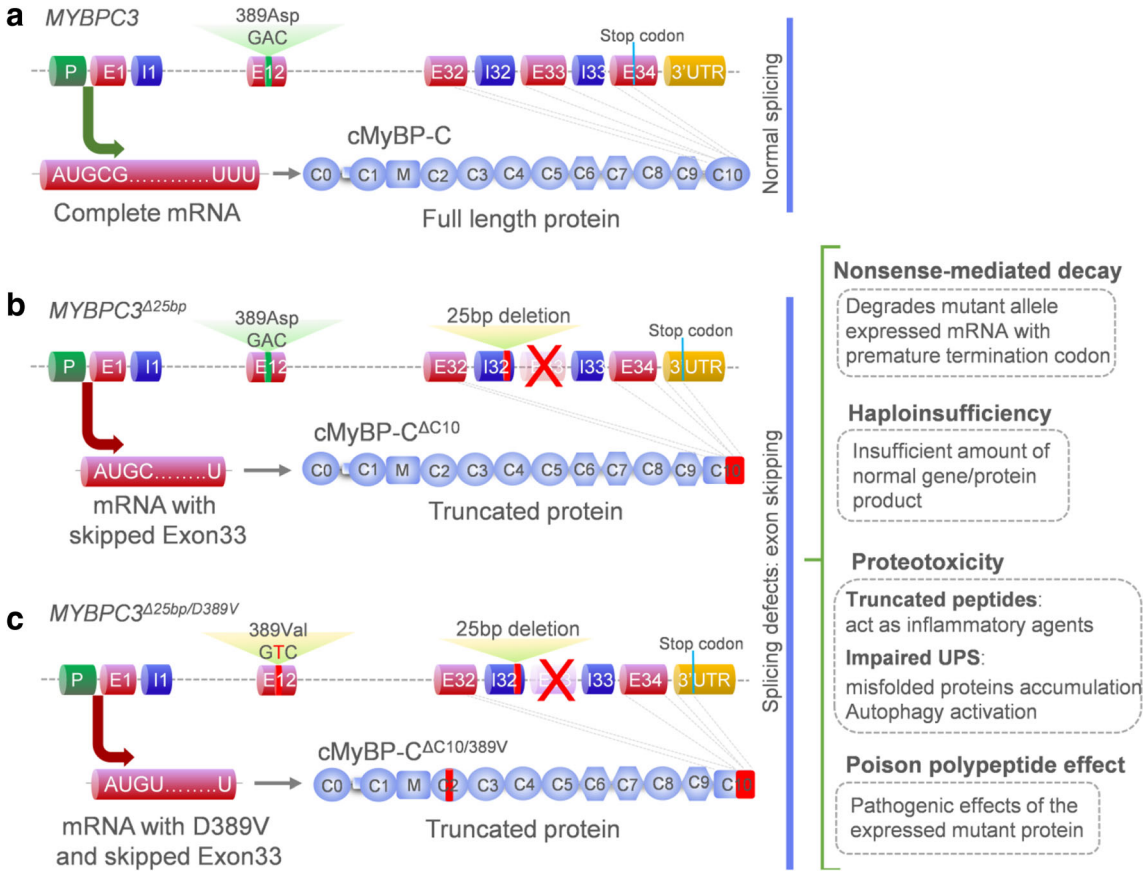

Fig. 5 *MYBPC3*$^{\Delta 25bp}$ and *MYBPC3*$^{\Delta 25bp/D389V}$ variants and associated pathogenesis. (**a**) Schematic illustration showing transcription of non-carrier *MYBPC3* allele coding normal cMyBP-C protein. (**b**) Intron 32 carrying *MYBPC3*$^{\Delta 25bp}$ mutation drives splicing defect causing exon 33 skipping and coding mutated C10 domain (cMyBP-C$^{\Delta C10mut}$). (**c**) Similarly, an additional point mutation, i.e., D389V, along with *MYBPC3*$^{\Delta 25bp}$ (together represented as *MYBPC3*$^{\Delta 25bp/D389V}$), is also presented. Such mutations drive abnormal *MYBPC3* gene transcription and translation leading to diverse processes/pathways that have adverse effects on cardiomyocyte health that may result in cardiomyopathy phenotype

possibility, a recent study of mouse cardiomyocytes with cMyBP-C haploinsufficiency showed that treatment with the myosin inhibitor mavacamten improved super-relaxed myosin to contractile function (Toepfer et al. 2019b), suggesting a clear relationship between cMyBP-C haploinsufficiency and contractile dysfunction during HCM.

Poison polypeptide effect

Another possible pathological mechanism in HCM is the "poison polypeptide" effect, in which the mutant proteins, either missense or truncated, cause dysfunction through integration into the sarcomere (Knoll 2012). In the case of cMyBP-C$^{\Delta C10mut}$, improper assembly of the cardiac sarcomere can occur, resulting in the development of contractile dysfunction and HCM (Kuster et al. 2019). Using transgenic mice expressing the cardiac-specific cMyBP-C$^{\Delta C10mut}$ protein, Kuster et al. demonstrated that cMyBP-C$^{\Delta C10mut}$ protein does not incorporate into its native sarcomeric C-zone. Instead, it preferentially localizes to the cytosol and Z-line. Increased toxic mutant and truncated proteins, if the poisoning process is operative, result in altered contractile function, thereby altering calcium handling and inducing pro-hypertrophic signaling in the cardiomyocytes that express cMyBP-C$^{\Delta C10mut}$ proteins. Both in vitro (Kuster et al. 2015) and in vivo (Kuster et al. 2019) experiments have concluded that cMyBP-C$^{\Delta C10mut}$ could be expressed in the cardiomyocytes, which would have turned into "poison polypeptide" effects on cardiomyocyte contractility. When cMyBP-C$^{\Delta C10mut}$ is expressed and localized improperly, it could alter the speed of contraction by regulating actomyosin interactions as the amino terminus of cMyBP-C$^{\Delta C10mut}$ is unaltered (Razzaque et al. 2013; Witayavanitkul et al. 2014). However, this hypothesis remains speculative by the failure of several studies to detect the mutant cMyBP-C proteins in the biopsies of patients with HCM (Jacques et al. 2008; Knoll 2012; van Dijk et al. 2009).

Proteotoxicity: inflammation and impaired ubiquitin-proteasome system

Several reports have suggested that *MYBPC3* mutations could produce toxic effects and that truncated proteins induce

inflammatory proteotoxicity and HCM (Bahrudin et al. 2011; Gupta et al. 2014; Schlossarek et al. 2014; Wang and Robbins 2014). More recently, Lipps et al. showed that cMyBP-C is degraded and released into myocardial tissue as a predominantly N-terminal 40-kDa fragment (C0C1f) with pro-inflammatory characteristics in the systemic circulation (Lipps et al. 2016). Alternatively, cMyBP-C$^{\Delta C10mut}$ could involve impairment of the ubiquitin-proteasome system (UPS) (Bahrudin et al. 2008; Sarikas et al. 2005). Eukaryotic cells use the lysosomal pathway (autophagy) (Bhuiyan et al. 2013; Maloyan and Robbins 2010; Pattison and Robbins 2011; Wang and Robbins 2014) and the UPS to remove unwanted proteins from the cytosol (Bahrudin et al. 2011; Carrier et al. 2010; Predmore et al. 2010; Sarikas et al. 2005; Schlossarek et al. 2014). In theory, the elevated levels of mutant protein could overwhelm and impair the UPS (Bahrudin et al. 2008; Schlossarek et al. 2012). Consistent with this notion, HCM patients with sarcomeric mutations have been observed with reduced proteasome activity compared with patients without the sarcomeric mutation (Predmore et al. 2010). Moreover, improper functioning of UPS may contribute to the disease process via (1) accumulation of malfunctioning proteins like cMyBP-C and (2) aggregated formation of unfolded proteins. Because cardiac UPS system efficiency is reduced with aging (Bulteau et al. 2002), we propose that disease penetrance in *MYBPC3*$^{\Delta 25bp}$ carriers is determined by the level of UPS impairment, which could be age-dependent (Dhandapany et al. 2009). It is also possible that *MYBPC3* mutations destabilize the UPS system by accumulating more in the cytosol, further contributing to cardiomyocyte dysfunction (Sarikas et al. 2005). Finally, considering the well-described decline in process and pathways of the UPS with increasing oxidative stress and secondary risk factors, such as age (Bulteau et al. 2002; Okada et al. 1999), mutant cMyBP-C proteins may store in the cardiomyocyte, affect cellular homeostasis, activate autophagy, and cause LV dysfunction (Kumar et al. 2016). This is supported by recent studies in cMyBP-C$^{\Delta C10mut}$ transgenic mouse heart showing differential expression of several genes associated with proteasome function (Kuster et al. 2019), suggesting the direct association among *MYBPC3*$^{\Delta 25bp}$, UPS impairment, and pathologic cardiac remodeling, leading to cardiomyopathy.

The endoplasmic reticulum (ER) and unfolded protein response (UPR) pathway regulate cellular homeostasis by mediating protein synthesis, folding, and maturation (Glembotski 2008; Liu and Dudley Jr 2014). The ER lumen contains three parallel signaling transmembrane sensor proteins, namely PRKR-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring protein 1α (IRE1α), all of which detect signs of ER stress in the cytosol and selectively recognize the accumulation of unfolded and misfolded proteins (Groenendyk et al. 2010; Minamino and Kitakaze 2010; Minamino et al. 2010). In the absence of ER stress, glucose-regulated protein 78 (GRP78, or BiP) binds and inhibits these three pathways. However, when misfolded proteins accumulate, GRP78 translocates from the ER lumen by dissociating from PERK, IRE1α, and ATF6, thereby activating those pathways. Accumulation of unfolded and misfolded proteins in the cell results in ER stress and activates PERK by autophosphorylation. Activated PERK, in turn, phosphorylates eukaryotic initiation factor 2α (eIF2α), which reduces cellular translation by ATF4 and CHOP activation (Halliday et al. 2014; Kitakaze and Tsukamoto 2010; Thorp 2012). PERK activation promotes apoptosis by inhibiting translational machinery, inducing the pro-apoptotic proteins Bax and Bim, and preventing the function of antiapoptotic protein Bcl2. Conversely, the activation of IRE1α and ATF6 induces cell survival by up-regulating ER chaperones. Furthermore, the accumulation of high-molecular-weight cMyBP-C$^{\Delta C10}$ proteins in the cytoplasm might alter the proteolytic capacity of the UPS and limit the ability of proteasomes to degrade other substrates. If shown, the results could elucidate novel therapeutic targets to augment the UPR pathway, as well as proteasome activity, and maintain sarcomere integrity, thereby attenuating the numerous types of heart disease linked to proteotoxicity. Therefore, it is possible that preventing activation of the PERK-mediated pathway in cMyBP-C$^{\Delta C10mut}$ expression would prevent or rescue the HCM phenotype (Groenendyk et al. 2010).

Nonsense-mediated decay

Nonsense-mediated decay (NMD) is a cellular process in the cytosol that detects mRNA with nonsense mutations and prevents their translations to mutant proteins. Specifically, NMD degrades mRNAs with premature termination codons (PTCs). NMD performs a dual role as it not only degrades PTC-containing transcripts but also regulates the expression of normal transcripts. HCM-associated *MYBPC3* mutations are predominantly premature stop codons. As such, the NMD pathway is the preferred pathway to remove them from the cellular process (Schlossarek et al. 2011; Vignier et al. 2009). Interestingly, using human-induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs), Seeger et al. demonstrated that *MYBPC3* PTC mutations involve abnormal calcium signaling and molecular dysregulation in the absence of significant haploinsufficiency of cMyBP-C protein (Seeger et al. 2019). Also, modulation of the cMyBP-C degradation pathway through therapeutic approaches may protect normal levels of cMyBP-C in HCM patients (Helms et al. 2020). Also, acute inhibition of NMD leads to a restoration of the HCM phenotype in hiPSC-CMs in vitro. Demonstration of such a direct relationship between NMD system activity and HCM development thus challenges haploinsufficiency or poison peptide mechanism as likely causes of *MYBPC3* PTC-mediated HCM (Seeger et al. 2019). We believe that the

NMD could remove and prevent mutant *MYBPC3*$^{\Delta 25bp}$ mRNA exon 33 skipping or alternative splicing to translate into cMyBP-C$^{\Delta C10mut}$ protein, leading to haploinsufficiency and causing contractile dysfunction, left ventricular hypertrophy, and HF.

Impact of MYBPC3$^{\Delta 25bp}$ with compound mutations

Not all carriers of the genetic variant *MYBPC3*$^{\Delta 25bp}$ show a cardiomyopathic phenotype (Dhandapany et al. 2009; Viswanathan et al. 2018). Cardiomyopathy is more likely and more pronounced when *MYBPC3*$^{\Delta 25bp}$ exists with other sarcomeric gene mutations (Bashyam et al. 2012; Tanjore et al. 2008; Waldmuller et al. 2003). For example, patients carrying both *MYBPC3*$^{\Delta 25bp}$ and another mutant in *MYH7-E927del* present with a severe HCM disease (Waldmuller et al. 2003). Likewise, when *MYBPC3*$^{\Delta 25bp}$ coexists with *TNNT2* mutants, HCM appears to be more likely (Kumar et al. 2016). Although heterozygous *MYBPC3*$^{\Delta 25bp}$ carriers predominantly present with incomplete penetrance and develop HCM symptoms at late-onset (Dhandapany et al. 2009), a compound effect of *MYBPC3*$^{\Delta 25bp}$ with other sarcomeric gene mutations (termed "two-hit phenomenon") can cause severe HCM and early sudden death (Marian 2001; Morita et al. 2008; Srivastava et al. 2011) and CAD (Kumar et al. 2016). Homozygous carriers of this *MYBPC3*$^{\Delta 25bp}$ variant, which is an example, in principle, of "two hits" can develop severe HCM phenotype and early clinical symptoms (Dhandapany et al. 2009).

Recent studies by our group show an association of cardiomyopathy/HCM with *MYBPC3*$^{\Delta 25bp}$ and *MYBPC3*^{D389V} mutations in SA descendants (Viswanathan et al. 2018) (Fig. 5). SAs living in the USA have a ~6% prevalence of the *MYBPC3*$^{\Delta 25bp}$ variant (Viswanathan et al. 2018); ~9.6% of these individuals also harbor a unique missense mutation (aspartic acid (D) to valine (V) at codon 389 in cMyBP-C) represented as *MYBPC3*^{D389V}. This variant is located at exon 12 which codes for the C2 domain of cMyBP-C. The C2 domain is a critical region of cMyBP-C that directly interacts with the S2 region of myosin in a phosphorylation-dependent manner and thereby regulates actomyosin and speed of contractions (Ratti et al. 2011). Interestingly, *MYBPC3*^{D389V} coexists with the *MYBPC3*$^{\Delta 25bp}$ variant in the same allele. The combined impact of both mutations predicts changes in actin-myosin interaction, abnormal Ca^{2+} transients, and cardiac dysfunction (heart hyperdynamic state) predisposing to HCM/HF (Viswanathan et al. 2018). Because *MYBPC3*^{D389V} is linked to HCM, it is proposed that *MYBPC3*^{D389V} is translated and incorporated into the sarcomere to exert HCM. For instance, if a carrier of the *MYBPC3*$^{\Delta 25bp}$ variant also bears a mutation in *MYH7*, this grouping often results in sudden cardiac death (Dhandapany et al. 2009). Systematic studies are needed to define whether *MYBPC3*$^{\Delta 25bp}$ alone is sufficient to cause LV dysfunction, LVH, and HCM or whether a definite association with another mutation or a secondary risk factor is needed (Van Driest et al. 2004).

. Compound mutations with *MYBPC3*$^{\Delta 25bp}$ may also cause cardiac arrhythmias, leading to the likelihood of organ damage, organ failure, and SCD (Al-Khatib et al. 2018; Hunt et al. 2001). More than 4 million Americans suffer from recurrent arrhythmias, and this statistic is expected to increase as the population ages. Almost 40% of all patients seen in cardiology clinics, including one in four seen for the first time, present with cardiac arrhythmia or conduction defect (Vazquez Ruiz de Castroviejo et al. 2005). Current medical therapies for treating cardiac arrhythmias suppress neurohormonal activation first and then treat cardiac fluid volume overload and function. It has been proposed that antiarrhythmic drugs might slow the progression of HCM. Nevertheless, the prognosis for patients, including those receiving optimal treatments, remains poor (Bristow et al. 1996; Coats 2002; Colucci et al. 1996)These circumstances call for the development of novel therapies for cardiac arrhythmia (Bristow 2000; From 1998; Sabbah and Stanley 2002; Tang and Francis 2003). Cardiac arrhythmias such as tachycardia, ventricular and atrial fibrillation, and premature contractions) are the major causes of SCD in HF (Swaminathan et al. 2012). Arrhythmias also lead to the worsening of HF by decreasing the efficiency of the whole organ and disrupting the link between heart rate and cardiac output. Unfortunately, certain treatments for HF, such as inotropic drugs that change the force of the heart's contractions, are contraindicated owing to increased SCD events (Krell et al. 1986), while conventional antiarrhythmic treatments, like Ca^{2+} channel blockers, cannot be used in HF owing to the worsening function of the failing myocardium with use of this class of drugs (Echt et al. 1991). Therefore, notwithstanding the association between cardiac arrhythmias and HF, it is difficult to strategize and develop novel therapies that can be used to treat both diseases without any side effects. In this process, the important first step is to identify novel therapeutic targets that can reduce HCM-induced cardiac arrhythmias and HF. Future studies should involve the elucidation of molecular mechanisms that cause the abnormal Ca^{2+} handling in HCM and SCD. We propose that the confluence of modifiers, including calcium handling proteins, such as phospholamban, sarco/endoplasmic reticulum Ca^{2+}-ATPase, ryanodine receptor (RyR), or histidine-rich Ca^{2+}-binding protein (HRC) with *MYBPC3*$^{\Delta 25bp}$ variant, could contribute to the development and manifestation of a severe cardiomyopathy phenotype and SCD, indicating that a more complex genetic architecture is involved in the disease (Wu et al. 2015).

Epigenetic changes and MYBPC3$^{\Delta 25bp}$

Epigenetics means "on top of" or "in addition to" the traditional genetic features involved in gene regulation. Epigenetics involves DNA methylation, histone modifications, and RNA-mediated silencing. DNA methylation, such as methylcytosine and hydroxymethylcytosine, occurs in cytosine nucleotides at CpG sites specifically linked to guanine by a phosphate. Utilizing an *MYBPC3* mutant mouse model of cardiomyopathy, Tabish et al. reported significant epigenetic changes within the introns of several cardiac genes associated with pathological cardiac remodeling and heart contractile dysfunction (Tabish et al. 2019). Based on these data, epigenetic changes in the intronic region of *MYBPC3* may induce altered splicing causing exon skipping and the rise of *MYBPC3*$^{\Delta 25bp}$ variant. Similarly, some patients within the same family cohort carrying an *MYBPC3*$^{\Delta 25bp}$ variant show HCM phenotype at the age of 30 years, while other carriers have healthy hearts, even in old age (Dhandapany et al. 2009). Others and we have hypothesized that the difference in phenotype could be explained by the epigenetic impact of comorbidities, such as promoting exon skipping or increasing alternative splicing. In addition, it has been suggested that HCM is directly associated with hypertension (Karam et al. 1989), a condition well known to exert epigenetic effects (Arif et al. 2019). Thus, the *MYBPC3*$^{\Delta 25bp}$ variant–related occurrence of HCM phenotype in patients at different ages and MetS may be classified by differences in epigenetic maps that activate and promote exon 33 skipping and increase alternative splicing. Such a notion may also determine the mechanism behind the differences in severity and onset of HCM at different ages in *MYBPC3*$^{\Delta 25bp}$ carriers.

Diagnosis and innovative therapeutic approaches

As mentioned earlier, one of the key conundrums of research in *MYBPC3*$^{\Delta 25bp}$ is incomplete penetrance and variable expressivity (Dhandapany et al. 2009; Waldmuller et al. 2003). The challenge is, in part, addressed by improving screening methods to detect asymptomatic cardiac disease. Diagnosis of HCM using contemporary technologies, such as magnetic resonance imaging, PCR, and biomarkers, is still in its early stages.

Next-generation sequencing–based strategies

Recent advances in DNA sequencing, such as next-generation sequencing (NGS) screening for genetic variants, are robust and high-throughput (Gomez et al. 2014), resulting in the identification of many new variants, as well as establishing new HCM databases. Such resources would help physicians make timely diagnostic decisions and start treatment (McNally and Puckelwartz 2015). The future of precision medicine will rely on genetic testing, genetic counseling, and therapeutic decisions based on collaborative efforts. Whole-genome DNA sequencing allows for the identification of multiple mutations within the same individual. Given the phenotypic implications of the association of *MYBPC3*$^{\Delta 25bp}$ with other gene mutations, such as *MYH7*, *TNNT2*, or *TNNI3*, this constitutes a major advance. The importance is emphasized by the understanding that mutations in both *MYBPC3* and *MYH7* consist about of 80% of the identified HCM-associated mutations, while another 10% are derived from *TNNI3* and *TNNT2* genes (McNally et al. 2015; Morimoto 2008). Therefore, at least 90% of HCM cases caused by mutations in these four major sarcomeric genes can be diagnosed via the design of an NGS panel. In fact, recent technological developments enabled researchers to use NGS as a molecular diagnostic tool for HCM (Chen et al. 2019) and other cardiomyopathies (Daoud et al. 2019; Forleo et al. 2017). In 2016, Barefield and coworkers performed a pilot study to develop a variant-specific diagnostic assay and quickly screen for the presence of the *MYBPC3*$^{\Delta 25bp}$ variant using either a small volume of blood or saliva from a cohort of SAs (Barefield et al. 2016). The RNaseH-mediated qPCR assay was used to diagnose the *MYBPC3*$^{\Delta 25bp}$ gene-positive with specific RNaseH-blocked nucleotide primers. Such assay provides faster prescreening of SAs who are at a high risk for cardiomyopathies with *MYBPC3*$^{\Delta 25bp}$ for earlier clinical care. Likewise, other non-sarcomeric gene mutations, such as *ACE* and extracardiac disease, can also be associated with the onset of HCM (Kraker et al. 2016); nevertheless, this is beyond the scope of this review.

Gene-editing technologies

Gene-editing technologies can be used to cure genetically caused HCM (Strong and Musunuru 2017). Clustered, regularly interspaced, short palindromic repeats (CRISPR) and CRISPR-associated 9 (Cas9) can provide specific site-directed gene editing. These technologies can also correct individual and selective gene mutations. Once such a system is validated, it could be an ideal means to treat patients with a homozygous *MYBPC3*$^{\Delta 25bp}$ variant. Recent studies show that the *MYBPC3* mutation can be corrected using the CRISPR/Cas9 in human pre-implanted embryos and that such non-carrier embryos, without evidence of off-target mutations, could be candidates for in vitro fertilization (Ma et al. 2017). However, in addition to possible ethical issues, it should be noted that the CRISPR/Cas9 system has some major technical limitations, e.g., ineffectiveness for repairing homozygous mutations or the possibility of off-targeting editing which affects other genes and thus induces secondary phenotypes.

Stem cell technologies: hiPSCs, spheroids, organoids, and engineered heart

Limitations in the availability of human cells and tissues present considerable obstacles to the study of human mutations. Furthermore, cardiomyocytes, a post-mitotic cell type, are almost impossible to maintain under culture conditions, leading to the inability to study such cells in vitro. A solution is a use of human-induced pluripotent stem cell (HiPSC) technology, a method that enables the use of renewable patient-specific biological material (Shi et al. 2017). The hiPSC system provides a unique opportunity to study genetic disease in vitro. These hiPSC-derived cardiomyocytes (hiPSC-CMs) are an ideal in vitro tool for testing drug cardiotoxicity, screening, and validation. HiPSCs can be differentiated into 2D cardiomyocytes (Kamdar et al. 2015), providing researchers the opportunity to study the importance of sarcomeric gene mutation and associated mechanisms of cardiomyopathy. For example, hiPSCs-CMs have been successfully utilized to study genetic diseases, including Pompe disease (Huang et al. 2011) and LEOPARD syndrome (Carvajal-Vergara et al. 2010). Similarly, the impact of MYBPC3 gene mutations has been studied in hiPSC-CMs (Helms et al. 2020; Ross et al. 2016; Seeger et al. 2019; Tanaka et al. 2014). However, HiPS-CMs have several limitations including incomplete reprogramming, an immature adult phenotype with embryonic genetic and epigenetic markers, variabilities, lack of t-tubules, and normal contractility (Knollmann 2013). Therefore, alternative and improved in vitro models are warranted. HiPS-CMs, cultured in a three-dimensional (3D) matrix architecture, termed as "spheroids" (Campbell et al. 2019) or "organoids" (Hoang et al. 2018), is another advancement in the field of cardiac tissue research. More importantly, these small 3D units under in vitro culture can be utilized as disease models (Ho et al. 2018; Mattapally et al. 2018). Another recent breakthrough in the field of cardiac research is the whole organ bioprinting using 3D tissue printing (Noor et al. 2019). Such pioneering research could allow cardiac studies to advance to a level that facilitates the investigation of cardiomyocyte and non-cardiomyocyte crosstalk and physiological function in a complex cardiac tissue–like environment. Recent developments in the use of hiPSCs differentiated into cardiomyocytes have allowed the fabrication of 3D heart muscle and cardiac organoids. Modern cell biology techniques have led to the generation of iPSC-derived cardiac organoids, resulting in a valuable approach toward assessing disease mechanisms and phenotype in vitro. Indeed, for recapitulating genetic diseases in Petri dishes, such as HCM, cardiac organoids may be essential. In particular, in vitro cardiac organogenesis using hiPSCs may lead to new opportunities for high-throughput modeling to study disease phenotype, determine disease mechanism, and perform preclinical studies to screen for therapeutic reagents. Such cardiac organoids may provide a tool with which to study organ development, cardiac function, and cardiac injury. HiPSCs-derived cardiac organoids may be considered a potential method to validate and assess disease phenotype and preclinical studies in a quick turnaround time and closely resemble human disease conditions, compared with hiPSC-CMs and mouse models (Marti-Figueroa and Ashton 2017; Silva et al. 2019). Organogenesis from hiPSCs may also provide new opportunities to study disease phenotype, define disease mechanisms, and help in screening for therapeutic reagents (Li et al. 2014). HiPSCs are now used to generate cardiomyocytes and engineer tissue, including 3D heart muscle and cardiac organoids (Mills et al. 2019; Mills et al. 2017). These cardiac organoids can act as a mechanical tool to study cardiac development, cardiac function, and cardiac injury (Moretti et al. 2013; Richards et al. 2017).

Gene therapies and pharmacological inhibitors to treat HCM

The percentage of HCM patients with *MYBPC3* mutations is estimated to be ~40%, making it the most common HCM-associated gene. Importantly, 70% of all MYBPC3 mutations are predicted to result in incomplete, or truncated, proteins, that alter sarcomere structure and function and impact cardiac mechanics (Barefield et al. 2015; Harris et al. 2011; Kuster et al. 2015; Sadayappan and de Tombe 2014). Recent drug development has focused on direct manipulation of the contractile apparatus by affecting myosin activators (Cleland et al. 2006; Solaro 2009; Teerlink 2009), modification of single histidine buttons in cardiac troponin I (cTnI) (Day et al. 2006; Palpant et al. 2009), the introduction of therapeutic genes via the expression of full-length cMyBP-C (Mamidi et al. 2014; Mearini et al. 2014; Merkulov et al. 2012), or the removal of mutant myosin transcripts (Jiang et al. 2013). Mearini et al. used AAV9-mediated expression of full-length cMyBP-C to improve haploinsufficiency and affect polypeptide expression. However, this study was limited by the fact that AAV9-mediated cMyBP-C was administered in 1-day-old mice to prevent HCM, rather than testing in adult mice once the HCM phenotype had been established (Mearini et al. 2014), or partially rescued. Alternatively, in vitro systems, such as hiPSC-derived cardiomyocytes (Prondzynski et al. 2017) and engineered heart tissues (Dutsch et al. 2019), have been used to test the efficacy of gene therapy designed to improve *MYBPC3* mutant–mediated contractile dysfunction; however, such systems require further in vivo validation using preclinical models (Prondzynski et al. 2019).

Direct targeting of contractile proteins, effectively bypassing receptor signaling, could lead to more promising therapeutic results (Day et al. 2006; James et al. 2005; Sadayappan et al. 2009; Solaro 2009; Teerlink 2009; Tissier et al. 2008). Unfortunately, most pharmacologic options

available today at best only modestly improve symptoms associated with HF. However, Ho et al. have demonstrated that diltiazem, a calcium channel blocker used to treat hypertension, also prevents the development of HCM in patients with *MYBPC3* mutations (Ho et al. 2015). A recently studied alternative to normalizing actomyosin interactions by manipulating signaling cascades is direct inactivation or activation of myosin (Mamidi et al. 2018). Such drugs might be prescribed to family members at risk at an earlier time before they develop a functional reduction of cardiac output. This approach has largely been directed toward improving cardiac function in inherited disease featuring diastolic dysfunction, namely restrictive cardiomyopathy (Davis et al. 2007) and HCM (Green et al. 2016; Kawas et al. 2017). Mavacamten, a myosin inhibitor, was established to treat HCM with left ventricular hypertrophy, disorganized sarcomere, fibrosis, and diastolic dysfunction. Early research has shown that chronic administration of mavacamten reduces hypercontractility in LVH, as well as disarray and fibrosis in the myocardium, and normalizes gene expression to rescue HCM in a mouse model (Green et al. 2016). In addition, Mamidi et al. demonstrated that Myk461-induced force reduction is regulated via cMyBP-C expression levels in the sarcomere (Mamidi et al. 2018). Mavacamten is a very promising drug to treat HCM and was recently approved for use in clinical trials (Phase 3 pivotal EXPLORER-HCM clinical trial, NCT03470545) for HCM (Heitner et al. 2019). Recently, mavacamten was also used to improve the hiPSC-CM function carrying a heterozygous *MYBPC3* mutation in vitro (Toepfer et al. 2019a). Based on this information, molecular inhibitors seem to have the potential to target the HCM phenotype, yet more focused studies to design relevant future therapies are needed. Thus, direct manipulation of the contractile apparatus is now a focus on drug development and could have significant therapeutic advantages by bypassing receptor-ligand signaling pathways (Cleland et al. 2006).

Concluding remarks

In SAs, $MYBPC3^{\Delta 25bp}$ is specifically inherited with an increased, but poorly understood, risk of HCM, HF, and sudden death. The high prevalence of $MYBPC3^{\Delta 25bp}$ and associated risk of cardiomyopathy have prompted urgency for understanding pathophysiology as a means to improve outcomes. This review highlights some of the mechanisms that may explain the association of $MYBPC3^{\Delta 25bp}$ with HCM, HF, and sudden death. Also, we summarize some of the current tools and techniques that may be helpful for the development of precision medicine to address the clinical problem of HCM.

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Compliance with ethical standards

Conflict of interest Dr. Sadayappan provides consulting and collaborative research studies to the Leducq Foundation (CURE-PLAN), Red Saree Inc., Greater Cincinnati Tamil Sangam, AstraZeneca, MyoKardia, Merck, and Amgen, but such work is unrelated to the content of this manuscript. Dr. Becker serves on scientific advisory boards for following: Janssen and DSMB Committees for Ionis Pharmaceuticals, Akcea Therapeutics, and Novartis. Dr. Singh has been a post-doctoral fellow of Amgen, starting from June, 2019 and performs research at the University of Cincinnati. No other disclosures are reported.

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