Gene 573 (2015) 188–197

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Gene wiki review

Cardiac myosin-binding protein C (*MYBPC3*) in cardiac pathophysiology

Lucie Carrier *, Giulia Mearini, Konstantina Stathopoulou, Friederike Cuello

[a] Department of Experimental Pharmacology and Toxicology, Cardiovascular Research Center, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[b] DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Hamburg, Germany

ARTICLE INFO

Article history:
Received 28 May 2015
Received in revised form 21 August 2015
Accepted 1 September 2015
Available online 8 September 2015

Keywords:
MYBPC3
Cardiac myosin-binding protein C
Cardiomyopathies
Hypertrophy postranslational modifications
Gene therapy

ABSTRACT

More than 350 individual *MYPBC3* mutations have been identified in patients with inherited hypertrophic cardiomyopathy (HCM), thus representing 40–50% of all HCM mutations, making it the most frequently mutated gene in HCM. HCM is considered a disease of the sarcomere and is characterized by left ventricular hypertrophy, myocyte disarray and diastolic dysfunction. *MYBPC3* encodes for the thick filament associated protein cardiac myosin-binding protein C (cMyBP-C), a signaling node in cardiac myocytes that contributes to the maintenance of sarcomeric structure and regulation of contraction and relaxation.
This review aims to provide a succinct overview of how mutations in *MYBPC3* are considered to affect the physiological function of cMyBP-C, thus causing the deleterious consequences observed in HCM patients. Importantly, recent advances to causally treat HCM by repairing *MYBPC3* mutations by gene therapy are discussed here, providing a promising alternative to heart transplantation for patients with a fatal form of neonatal cardiomyopathy due to bi-allelic truncating *MYBPC3* mutations.

Contents

Abbreviations: AAV, adeno-associated virus; AON, antisense oligonucleotide; bp, base pair; Ca^{2+}, calcium; CaMKII, Ca^{2+}/calmodulin-dependent kinase II; Chr, chromosome; CK2, casein kinase 2; *CMH4*, hypertrophic cardiomyopathy, locus 4; cMyBP-C, cardiac myosin-binding protein C; CRISPR, clustered regularly interspaced short palindromic repeats; DCM, dilated cardiomyopathy; EHT, engineered heart tissue; GSK3β, glycogen-synthase kinase isoform 3β; HCM, hypertrophic cardiomyopathy; iPSC, induced-pluripotent stem cell; k_{tr}, rate of force redevelopment; LMM, light meromyosin; LVNC, left ventricular non-compaction; M or M motif, myosin-binding protein motif; *Mybpc3*$^{-/-}$, myosin-binding protein C knockout; *MYBPC3*, human cardiac myosin-binding protein C gene; *Mybpc3*, mouse cardiac myosin-binding protein C gene; *MYH7*, human β-myosin-heavy chain gene; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PKD, protein kinase D; PTM, posttranslational modification; RNS, reactive nitrogen species; ROS, reactive oxygen species; *TNNT2*, human cardiac troponin T gene; *TPM1*, human α-tropomyosin gene; UPS, ubiquitin–proteasome system.

* Corresponding author at: Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany.
E-mail address: l.carrier@uke.de (L. Carrier).

http://dx.doi.org/10.1016/j.gene.2015.09.008
0378-1119/

1. Introduction

One of the first major steps in understanding the structure and function of *MYBPC3* (OMIM #600958), encoding cardiac myosin-binding protein C (cMyBP-C) has been the cloning of the human cDNA and localization of the gene on human chromosomal 11p11.2 by the group of Labeit (Gautel et al., 1995) (hg 19/GCRh37 reference chr11:47,353,396–47,374,253, and ENST00000545968, NM_000256.3). *MYBPC3* became therefore the "best" candidate gene for the *CMH4* locus for hypertrophic cardiomyopathy (HCM) that was previously mapped by the group of Schwartz (Carrier et al., 1993). Another major step has been the simultaneous identification of the first two *MYBPC3* mutations segregating in HCM families by the respective groups of Schwartz and Seidman (Bonne et al., 1995; Watkins et al., 1995). *MYBPC3* was thus chronologically the fourth HCM gene, following *MYH7*, encoding β-myosin heavy chain (Geisterfer-Lowrance et al., 1990), *TNNT2* and *TPM1*, encoding cardiac troponin T and α-tropomyosin, respectively (Thierfelder et al., 1994), earmarking HCM as a disease of the sarcomere. The evidence that *MYBPC3* defects result in an inherited cardiomyopathy brought back the interest in understanding the function of cMyBP-C in the cardiac sarcomere.

The structure and sequence of the human *MYBPC3* gene have been determined in 1997 (Carrier et al., 1997). It encompasses more than 21 kbp and is composed of 34 coding exons (Fig. 1). MyBP-C is a multi-modular structural protein component of the sarcomere. It decorates the C-zone of the A band, forming doublet appearing transverse stripes approximately 43 nm apart of each other in the cross-bridge bearing region (Fig. 2; Luther et al., 2008), reviewed by (Schlossarek et al., 2011). Three highly homologous isoforms of MyBP-C exist in adult human muscle: the slow-skeletal (encoded by the *MYBPC1* gene which resides on chromosome 12q23.3), the fast skeletal (encoded by the *MYBPC2* gene which resides on chromosome 19q33.3) and the cardiac isoform (cMyBP-C). The cardiac isoform is exclusively expressed in the heart during human and mouse development (Fougerousse et al., 1998). cMyBP-C consists of eight immunoglobulin-like and three fibronectin-like domains (Fig. 1). In comparison to the slow and fast skeletal isoforms, cMyBP-C has characteristic structural additions, making it an ideal platform for signaling. These include an additional immunoglobulin-like domain at the N-terminus (C0 domain), multiple phosphorylation sites in the MyBP-C motif (M motif) between the immunoglobulin-like domains C1 and C2 and a 28-amino acid insertion within the C5 domain (for reviews, see Carrier et al., 1998; Flashman et al., 2004; Barefield and Sadayappan, 2010; Schlossarek et al., 2011; Pfuhl and Gautel, 2012; Sadayappan and de Tombe, 2012).

2. *MYBPC3* and inherited cardiomyopathies

2.1. MYBPC3 mutations

2.1.1. MYBPC3 and hypertrophic cardiomyopathy

More than 350 individual *MYBPC3* mutations have been identified causing HCM and thus representing 40–50% of all HCM mutations, making it the most frequently mutated gene in HCM (for reviews, see Schlossarek et al., 2011; Behrens-Gawlik et al., 2014). HCM is a myocardial disease characterized by left ventricular hypertrophy, myocardial disarray and diastolic dysfunction (for reviews, see Authors/Task Force, members, Elliott et al., 2014; Olivotto et al., 2015), with an estimated prevalence based on hypertrophy of 1:500 in young adults (Maron et al., 1995). It is mainly transmitted in an autosomal-dominant fashion and associates with more than 1000 mutations in at least 10 genes, encoding components of the sarcomere (for reviews, see Ho et al., 2015; Olivotto et al,. 2015). Most of the known *MYBPC3* mutations (>60%) are truncating mutations, including nonsense mutations,

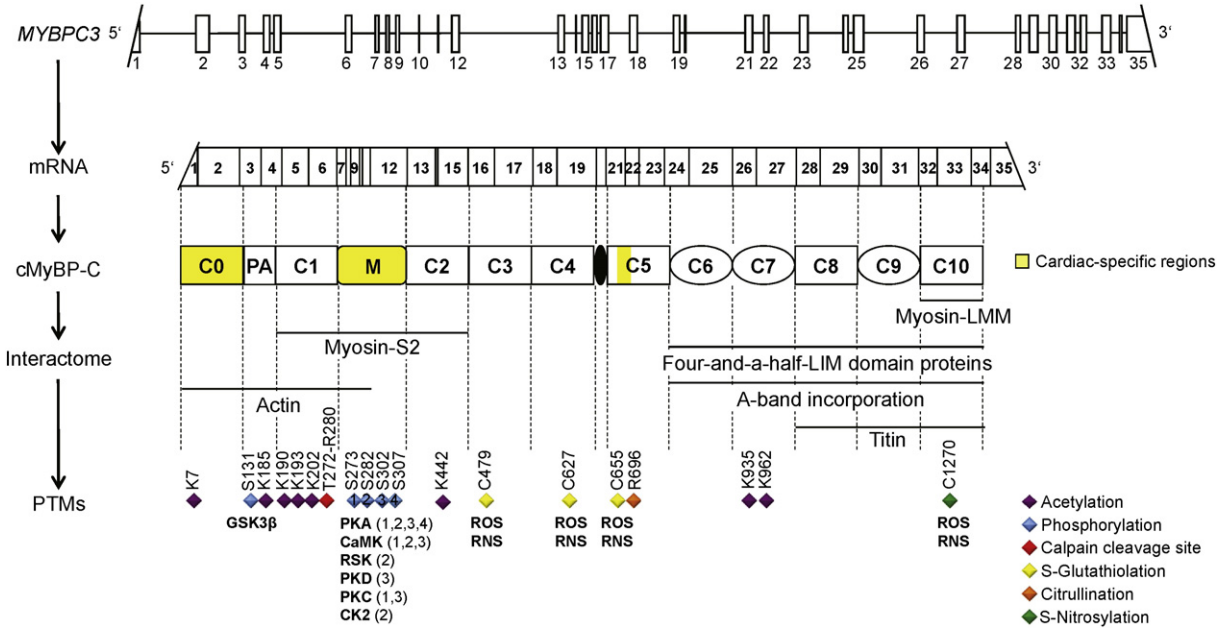

Fig. 1. Schematic representation of *MYBPC3* gene, mRNA and protein structure, its interaction partners (interactome) and sites of posttranslational modifications (PTMs). *MYBPC3* encompasses 21 kbp and is composed of 35 exons, which is transcribed into a 3824-bp transcript. cMyBP-C is a multi-modular protein composed of 8 immunoglobulin-like (C0, C1, C2, C3, C4, C5, C8, C10) and 3 fibronectin-type III (C6, C7, C9) domains. The cardiac isoform differs from the slow-skeletal and the fast skeletal isoforms by cardiac-specific regions (C0, M, 28-amino acid insertion in C5) that are highlighted in yellow. Between C0 and C1 exists a proline-alanine rich domain (Pro-Ala; PA). The linker between C4 and C5 domains is indicated by a black oval. Anchoring of cMyBP-C to the C-zones of sarcomeric A-bands is mediated by domains C6 to C10. The NH_2-terminus of cMyBP-C interacts with F-actin (C0-M) and the myosin-S2 domain (C1-M-C2). The COOH-terminus of cMyBP-C interacts with members of the four-and-a-half-LIM-domain family (C6–C10), titin (C8–C10) and light meromyosin (LMM; C10). cMyBP-C is subject to a variety of PTMs that are depicted with different colored diamonds for acetylation (purple), phosphorylation (blue), calpain cleavage (red), S-glutathiolation (yellow), citrullination (orange), and S-nitrosylation (green). Involved protein kinases are shown below the symbols. Abbreviations used: CaMK, Ca^{2+}/calmodulin-dependent protein kinase; CK2, casein kinase 2; GSK3β, glycogen-synthase kinase isoform 3β; M, MyBP-C motif; PKA, cAMP-dependent protein kinase; M, MyBP-C motif; PKD, protein kinase D; PKC, protein kinase C; RNS, reactive nitrogen species; ROS, reactive oxygen species; RSK, p90 ribosomal S6 kinase. Numbering of amino acids refers to the mouse sequence.

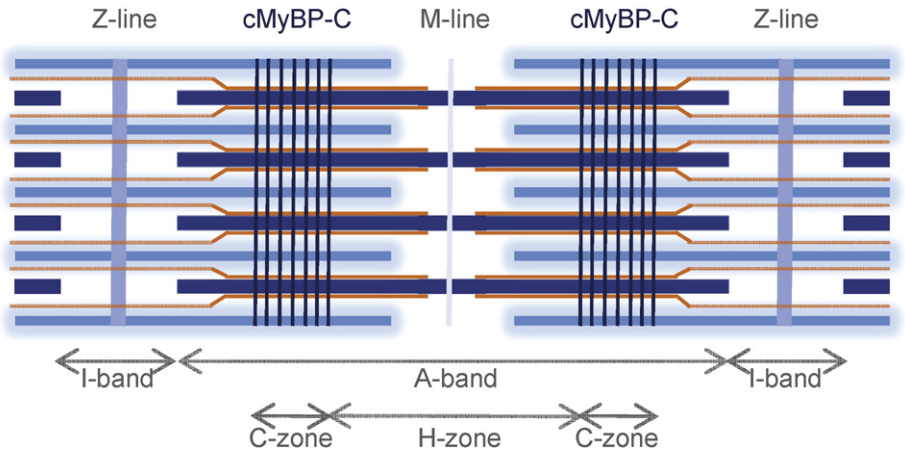

Fig. 2. Schematic diagram depicting sarcomeric localization of cMyBP-C. cMyBP-C (dark blue) localization is restricted to the C-zones of sarcomeric A-bands in 7–9 transverse stripes at both sites of the M-line. Thin filament (light blue), thick filament (middle blue), titin (orange).

insertions or deletions, splicing or branch point mutations, leading to COOH-terminally truncated cMyBP-C, lacking the major myosin- and/or titin-binding sites (for review, see Behrens-Gawlik et al., 2014). The majority of *MYBPC3*-associated HCM mutations are heterozygous, and patients often have a late disease onset with a benign disease progression.

2.1.2. *MYBPC3* and other cardiomyopathies

Genetic studies have revealed significant overlap between genotypes and phenotypes as *MYBPC3* mutations can lead to various forms of cardiomyopathies, such as dilated cardiomyopathy (DCM) and left ventricular non-compaction (LVNC), the latter being characterized by prominent trabeculation of the left ventricle and deep intertrabecular recesses (Hoedemaekers et al., 2010; Probst et al., 2011). Importantly, in a large European multi-centre, multi-national study enrolling a cohort of 639 patients with sporadic or familial DCM, *MYBPC3* mutations represented the second highest number of previously reported disease-causing mutations (38/294, i.e. 13%; (Haas et al., 2014)). Furthermore, a 25-bp deletion in *MYBPC3* intron 32 (original description Δ25) leading to a frameshift, initially associated with HCM in 2 families (Waldmuller et al., 2003) has been also found in 13.8% of patients with distinct inherited cardiomyopathies (including HCM, DCM and restrictive cardiomyopathy). This genetic variant is present in 4% of the population in South Asia and is associated with a 7-fold higher risk to develop heart failure (Dhandapany et al., 2009). Together, these studies reveal *MYBPC3* as one of the major mutated genes in cardiomyopathies and heart failure. Similar to HCM, both missense and truncating mutations were found in DCM and LVNC (Hoedemaekers et al., 2010; Probst et al., 2011; Haas et al., 2014).

2.1.3. Founder *MYBPC3* mutations in HCM

Founder *MYBPC3* mutations have been identified in some countries and populations, where they represent a large percentage of HCM cases (Table 1). Interestingly, all of them are truncating mutations, resulting in shorter cMyBP-C, lacking the phosphorylation M motif and/or major binding domains to other sarcomeric proteins (Table 1; Fig. 1). A founder c.927-2A>G transition in intron 11 that arose >550 years ago is the predominant cause (58%) of HCM in Iceland (Adalsteinsdottir et al., 2014). The c.912_913delTT (original description Int12ASA_2G; Niimura et al., 1998) mutation in exon 11 has been found in 19.5% of HCM probands in the Veneto region of Italy (Calore et al., 2015). In The Netherlands, 3 different founder *MYBPC3* mutations, c.2373_2374insG (or 2373dupG), c.2864_2865delCT and c.2827C>T were found in 17%, 2.6% and 1.6% of HCM cases, respectively (for review, see Christiaans et al., 2010). In Finland, the c.3183C>T transition (or Gln1061X) in exon 29 accounts for 17% of HCM cases (Jaaskelainen et al., 2004). In Japan, the c.1775delT (or Val592fs/8) in exon 18 was identified in 16% of HCM probands (Kubo et al. 2005). In Tuscany, the c.772G>A transition (original description Glu258Lys; Niimura et al., 1998) in exon 6 was found in 14% of HCM cases (Girolami et al., 2006). Finally, another report indicates that the c.1898-2A>G transition (original description IVS20-2; Bonne et al., 1995) in intron 20 appeared about 10 centuries ago and accounts for 8.4% of HCM patients in Center-East France (Teirlinck et al., 2012). Therefore, the likelihood to get

Table 1
Founder *MYBPC3* truncating mutations in HCM patients.

Reference	F[1]	Country	Mutation[2]	Original description	Location	Protein consequence[3]
Adalsteinsdottir et al. (2014)	58%	Iceland	c.927-2A>G	Int12ASA_2G	Intron 11	p.Pro309fsX26, PTC in exon 13, truncation in C2
Calore et al. (2015)	19.5%	Italy, Veneto	c.912-913delTT	delTT, F305fsd	Exon 11	p.Ser304fsX26, PTC in exon 12, truncation in M
Christiaans et al. (2010)	17%	The Netherlands	c.2373_2374insG	2373dupG	Exon 24	p.Trp792fsX17, PTC in exon 25, truncation in C6
Jaaskelainen et al. (2004)	17%	Finland	c.3183C>T	Gln1061X	Exon 29	p.Gln1061X, PTC in exon 29, truncation in C8
Kubo et al. (2005)	16%	Japan	c.1775delT	Val592fs/8	Exon 18	p.Val592fsX8, PTC in exon 19, truncation in C4
Girolami et al. (2006)	14%	Italy, Tuscany	c.772G>A	Glu258Lys	Exon 6	p.Glu258fsX41, PTC in exon 9, truncation in M
Teirlinck et al. (2012)	8.4%	France	c.1928-2A>G	IVS20-2	Intron 20	p.Gln643fsX2, PTC in exon 22, truncation in C5
Christiaans et al. (2010)	2.6%	The Netherlands	c.2827C>T	2827C>T	Exon 27	p.Arg943X, PTC in exon 27, truncation in C7
Christiaans et al. (2010)	1.6%	The Netherlands	c.2864_2865delCT	2864_2865delCT	Exon 27	p.Pro955fsX95, PTC in exon 29, truncation in C8

Abbreviations used: C2–C8, domains of cardiac myosin-binding protein C; F, frequency of the mutation; M, MyBP-C motif; fs, frameshift; HCM, hypertrophic cardiomyopathy; PTC, premature termination codon; Xn, PTC after n amino acids.

[1] Expressed as total number of cases with HCM.

[2] cDNA nomenclature, considering the A of ATG Met codon as 1.

[3] Protein nomenclature, considering ATG Met codon as 1.

compound heterozygotes or homozygotes with these truncating mutations in these countries is high.

2.1.4. Bi-allelic MYBPC3 mutations and pediatric cardiomyopathy

A body of evidence indicates that patients with more than 1 mutation often develop a more severe phenotype (Richard et al., 2003; Haas et al., 2014), and a significant fraction of childhood-onset HCM (14%) is caused by compound genetic variants (Morita et al., 2008). This suggests that a gene-dosage effect might be responsible for manifestations at a younger age. A total of 51 cases of homozygotes or compound heterozygotes have been reported, composed of 26 cases with double truncating mutations (Richard et al., 2003; Lekanne Deprez et al., 2006; Xin et al., 2007; Zahka et al., 2008; Ortiz et al., 2009; Tajsharghi et al., 2010; Marziliano et al., 2012; Schaefer et al., 2014; Wessels et al., 2014), 11 cases with a missense mutation plus a truncating mutation (Richard et al., 2003; Ingles et al., 2005; Morita et al., 2008; Dellefave et al., 2009; Saltzman et al., 2010; Otsuka et al., 2012), and 14 with double missense mutations (Nanni et al., 2003; Van Driest et al., 2004; Garcia-Castro et al., 2005; Ingles et al., 2005; Morita et al., 2008; Saltzman et al., 2010; Maron et al., 2012). These studies also showed that all patients with bi-allelic truncating *MYBPC3* mutations are associated with neonatal cardiomyopathy and lead to heart failure and death within the first year of life.

2.2. MYBPC3-associated pathomechanisms

A great understanding of how *MYBPC3* mutations lead to the development of inherited cardiomyopathy came from the analyses of human myocardial samples, gene transfer in different cell lines, naturally-occurring or transgenic animal models and more recently disease-modeling using induced-pluripotent stem cells (iPSC)-derived cardiac myocytes (for recent reviews, see Duncker et al., 2015; Eschenhagen et al., 2015). Although access to human myocardial samples is difficult, at least some studies provided evidence that truncated cMyBP-Cs, resulting from truncating *MYBPC3* mutations are not detectable in human patient samples by Western-blot analysis (Rottbauer et al., 1997; Moolman et al., 2000; Marston et al., 2009; van Dijk et al., 2009). This was supported in heterozygous *Mybpc3*-targeted knock-in mice (Vignier et al., 2009), carrying the human c.772G>A transition (i.e. founder mutation in Tuscany (Girolami et al., 2006); Table 1). These data suggest haploinsufficiency as the main disease mechanism for heterozygous truncating mutations (for reviews, see Marston et al., 2012; van der Velden et al., 2015). A body of evidence exists that the mechanisms regulating the expression of mutant allele involve the nonsense-mediated mRNA decay, the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway after gene transfer of mutant *MYBPC3* in cardiac myocytes or in mice *in vivo* (Sarikas et al., 2005; Bahrudin et al., 2008; Vignier et al., 2009), and for reviews (Mearini et al., 2008; Carrier et al., 2010; Schlossarek et al., 2014). In contrast to truncating mutations, missense mutations lead, in most of the cases (although difficult to specifically detect), to stable mutant cMyBP-Cs that are, at least in part, incorporated into the sarcomere and could act as poison polypeptides on the structure and/or function of the sarcomere. Homozygous or compound heterozygous mutations are therefore likely subject to differential regulation depending on whether they are double missense, double truncating or mixed missense/truncating mutations. The homozygous *Mybpc3*-targeted knock-in mice, which genetically mimic the situation of severe neonatal cardiomyopathy are born without disease phenotype and soon after birth develop systolic dysfunction followed by (compensatory) cardiac hypertrophy (Gedicke-Hornung et al., 2013; Mearini et al., 2013). The human c.772G>A transition results in low levels of three different mutant *Mybpc3* mRNAs and cMyBP-Cs in homozygous knock-in mice, suggesting a combination of haploinsufficiency and polypeptide poisoning as disease mechanism in the homozygous state (Vignier et al., 2009). The combination of external stress (such as neurohumoral stress or aging) and *Mybpc3* mutations have been shown to impair the UPS in mice (Schlossarek et al., 2012a, 2012b), and proteasomal activities were also depressed in patients with HCM (Predmore et al., 2010; Thottakara et al., 2015).

Skinned trabeculae or cardiac myocytes obtained from human patients carrying a *MYBPC3* mutation or from heterozygous and homozygous *Mybpc3*-targeted knock-in mice exhibited higher myofilament Ca^{2+} sensitivity than controls (Witt et al. 2001; van Dijk et al. 2009; Fraysse et al. 2012; van Dijk et al. 2012; Sequeira et al. 2013). Disease-modeling by engineered heart tissue (EHT) technology with cardiac cells from heterozygous or homozygous *Mybpc3*-targeted knock-in mice reproduced observations made in human and mouse studies displaying abbreviated contractions, greater sensitivity to external Ca^{2+} and smaller inotropic responses to various drugs (isoprenaline, EMD 57033 and verapamil) compared to wild-type control EHTs (Stohr et al. 2013). Therefore, EHTs are suitable to model the disease phenotype and recapitulate functional alterations found in HCM mice. Another good system for modeling cardiomyopathies in the cell culture dish is the derivation of cardiac myocytes from iPSC (for a recent review, see Eschenhagen et al. 2015). Four reports of human iPSC models of sarcomeric cardiomyopathies have been published in the last three years showing cellular hypertrophy in most of the cases (Sun et al. 2012; Lan et al. 2013; Han et al. 2014; Tanaka et al. 2014), including one with the c.2995_3010del *MYBPC3* mutation that exhibited in addition to hypertrophy contractile variability in the presence of endothelin-1 (Tanaka et al. 2014).

The mechanisms by which *MYBPC3* haploinsufficiency leads to increased myofilament Ca^{2+} sensitivity and the occurrence of a disease phenotype are not fully elucidated. It is obvious that all conditions result in a reduced amount or even complete absence of cMyBP-C (patients, knock-in or knockout mice, and after cMyBP-C extraction) and are associated with increased Ca^{2+} sensitivity of the myofilaments (Hofmann et al., 1991; Witt et al., 2001; Cazorla et al., 2006; van Dijk et al., 2009; Fraysse et al., 2012; van Dijk et al., 2012; Sequeira et al., 2013; Barefield et al., 2015). Since cMyBP-C has been shown to normally prevent residual crossbridge cycling at low cytosolic Ca^{2+} concentrations in diastole and thereby promote diastolic relaxation (Pohlmann et al., 2007), reduced or absent cMyBP-C could therefore help to explain diastolic dysfunction independent of hypertrophy as the early consequence of *MYBPC3* mutation in heterozygous patients and knock-in mice (Ho et al., 2009; Michels et al., 2009; Fraysse et al., 2012).

3. Lessons learnt from *Mybpc3*-targeted knockout (*Mybpc3*$^{-/-}$) mice

Indispensable information highlighting the importance of intact cMyBP-C for normal cardiac physiology has evolved from numerous studies in transgenic mice, in which the consequences of ablation of the protein (*Mybpc3*-targeted knockout mice; *Mybpc3*$^{-/-}$) were investigated.

Constitutive *Mybpc3*$^{-/-}$ mice are born in accordance with the Mendelian inheritance ratios, survive into adulthood and are fertile (Harris et al., 2002; Carrier et al., 2004). Three weeks from birth onwards, *Mybpc3*$^{-/-}$ mice develop severe cardiac hypertrophy with increased heart-weight-to-body-weight-ratios, enlargement of ventricles, increased myofilament Ca^{2+} sensitivity and depressed diastolic and systolic function (Harris et al., 2002; Carrier et al., 2004; Cazorla et al., 2006). Histologically, *Mybpc3*$^{-/-}$ hearts display structural rearrangements similar to those observed in explanted hearts from HCM patients with cardiac myocyte disarray and increased interstitial fibrosis, without obvious alterations in shape or size of single cardiac myocytes. Ultrastructural examination revealed a loss of lateral alignment of adjacent myofibrils with their Z-lines misaligned. Interestingly, an increased number of mitochondria of decreased size were noticed in *Mybpc3*$^{-/-}$ cardiac myocytes (Harris et al,. 2002; Carrier et al., 2004; Brickson et al., 2007; Luther et al., 2008). The first lesson from this mouse model learnt is that cMyBP-C is not essential for sarcomere formation

during embryogenesis, but is crucial for sarcomere organization and maintenance of normal cardiac function.

Studies performed in chemically permeabilized cardiac myocyte preparations from *Mybpc3*$^{-/-}$ mice further enlightened possible underlying molecular causes of the HCM phenotype in patients. Lack of cMyBP-C resulted in acceleration of crossbridge cycling rates as evidenced by increased loaded shortening and power output, as well as faster rates of force redevelopment (k_{tr}) at submaximal Ca^{2+} concentrations (Korte et al., 2003; Stelzer et al., 2006a, 2006b; Chen et al., 2010). This suggests that cMyBP-C regulates the positioning of myosin and actin for interaction and more precisely acts as to constrain actin-myosin interaction, which limits loaded shortening velocity and ultimately power output. This observation can be partially attributed to its N-terminal C1-M-C2 region interacting with the myosin-S2 domain (Gruen and Gautel, 1999; Kunst et al., 2000; Harris et al., 2004; Ababou et al,. 2007), which results in a decreased number of crossbridges formed, due to cMyBP-C acting as a tether to the myosin S1 heads, limiting their mobility (Weith et al., 2012) and thus leading to the observed functional consequences described above. Other studies with intact or skinned cardiac myocytes and atrial tissue from *Mybpc3*$^{-/-}$ mice have indicated that cMyBP-C contributes to the regulation of cardiac contraction at short sarcomere length and is required for complete relaxation in diastole (Cazorla et al., 2006; Pohlmann et al., 2007).

Several studies have suggested that the interactions of cMyBP-C with its binding partners vary with its phosphorylation status of the M motif, which contains multiple thoroughly characterized phosphorylation sites that are targeted all by cAMP-dependent protein kinase (PKA) and individually by a variety of other protein kinases (Hartzell and Titus, 1982; Hartzell and Glass, 1984; Gautel et al., 1995; Mohamed et al., 1998; McClellan et al., 2001; Haworth et al., 2004; Bardswell et al., 2010; Cuello et al., 2011; Sadayappan et al., 2011). In its dephosphorylated state, cMyBP-C binds predominantly to myosin S2 and brakes crossbridge formation, however, when phosphorylated in response to β-adrenergic stimulation through activating PKA, it favors binding to actin, then accelerating crossbridge formation, enhancing force development and promoting relaxation (Moss et al., 2015). Binding of the N-terminal C0-C1 region of cMyBP-C to actin (Kulikovskaya et al., 2003; Razumova et al., 2006; Rybakova et al., 2011; Weith et al., 2012) contributes to the sensitisation of the thin filament to Ca^{2+}, potentially through interaction with and therefore shift of tropomyosin into its high Ca^{2+} position, which exposes the myosin-binding sites on actin (Mun et al., 2014; Witayavanitkul et al., 2014; Previs et al., 2015). Three-dimensional electron-microscopy of intact sarcomeres with addition of cMyBP-C fragments confirmed that cMyBP-C associates indeed with both the thick and the thin filament (Zoghbi et al., 2008; Luther et al., 2011; Mun et al., 2011). Recently, a novel hypothesis for the physiological role of cMyBP-C in the regulation of cardiac contractility was postulated by Kampourakis and colleagues, who investigated structural changes of the thin and thick filaments after addition of recombinant C1-M-C2 region of cMyBP-C to chemically permeabilized contracting rat trabeculae (Kampourakis et al., 2014). They found that the N-terminus of cMyBP-C stabilized the ON state of the thin filaments and the OFF state of the thick filaments, leading to a model for the control of heart muscle contraction in which both filament systems are coordinated by cMyBP-C.

4. Phosphorylation and other "exotic" posttranslational modifications of cMyBP-C

Additionally to alterations in the protein content of cMyBP-C leading to haploinsufficiency and polypeptide poisoning in HCM, cMyBP-C function is also regulated by an ever-growing spectrum of posttranslational modifications (PTMs) with phosphorylation, acetylation, citrullination, and the oxidative PTMs S-glutathiolation, S-nitrosylation and carbonylation.

A large body of literature exists already on the phosphoregulation of cMyBP-C, which has been reviewed in depth elsewhere (Barefield and Sadayappan, 2010; Bardswell et al., 2012; Gupta and Robbins, 2014). In brief, at least three extensively characterized phosphorylation sites (Ser273, 282 and 302; numbering refers to the mouse sequence, Fig. 1) are localized in the M motif of cMyBP-C and are targeted by protein kinases in a hierarchical order of events starting with Ser282 that acts as a switch, rendering Ser273 and Ser302 more accessible for phosphorylation. Protein kinases identified thus far to phosphorylate cMyBP-C in the M motif are PKA (Gautel et al., 1995; Mohamed et al., 1998), Ca^{2+}/calmodulin-dependent kinase II (CaMKII; Sadayappan et al., 2011), p90 ribosomal S6 kinase (RSK; Cuello et al., 2011), protein kinase D (PKD; Haworth et al., 2004; Bardswell et al., 2010; Dirkx et al., 2012) and protein kinase C (PKC; Mohamed et al., 1998). Furthermore, GSK3β was described as another protein kinase to phosphorylate cMyBP-C outside the M-domain in the proline-alanine-rich actin-binding site at Ser133 in human myocardium (mouse Ser131; Kuster et al., 2013)). As described earlier, phosphorylation is required for normal cardiac function, and overall phosphorylation levels of cMyBP-C are reduced in human and experimental heart failure (El-Armouche et al., 2007; Copeland et al., 2010). Transgenic expression of a phosphomimetic cMyBP-C mutant protected hearts from ischemia-reperfusion injury (Sadayappan et al., 2005; Sadayappan et al., 2006) and reduced the appearance of a 40-kDa proteolytic calpain-degradation fragment of cMyBP-C (Govindan et al., 2012; Witayavanitkul et al., 2014). Taken together, phosphorylation of cMyBP-C is considered beneficial and preservation of phosphorylation combats disease progression. The question arises whether there are good and bad PTMs.

Acetylation was firstly reported by analyzing recombinant cMyBP-C by top-down high-resolution mass spectrometry (Ge et al., 2009) and was later confirmed *in vivo* in mouse hearts subjected to ischemia-reperfusion injury (Govindan et al., 2012). Eight acetylation sites were identified, five of which localized in the N-terminal C0–C1 region (Fig. 1). However, whether acetylation of cMyBP-C increases under disease conditions and whether acetylation is functionally important is not fully understood yet. Interestingly, the 40 kDa proteolytic calpain-degradation fragment (Thr272-Arg280) of cMyBP-C was found to be heavily acetylated (Govindan et al., 2012), suggesting that acetylation could promote proteolysis and decrease stability of cMyBP-C in contrast to phosphorylation.

Citrullination (Fert-Bober and Sokolove, 2014) of cMyBP-C was reported to occur, however it has not been functionally characterized with regards to its influence on cMyBP-C function. There is only limited information showing that cMyBP-C is citrullinated at Arg696 in both control and diseased heart tissue (Fert-Bober and Sokolove, 2014).

More importantly, cMyBP-C is also susceptible to oxidation such as S-glutathiolation, S-nitrosylation and carbonylation. S-glutathiolation, the formation of stable mixed disulfides between the sulfydryl groups of cysteinyl residues with glutathione (Dalle-Donne et al., 2008), was firstly reported as a reversible oxidative PTM of cMyBP-C in a screen of S-glutathiolated cardiac proteins using *N,N*-biotinyl glutathione disulfide (Brennan et al., 2006). Enhanced S-glutathiolation of a protein migrating at the same molecular mass as cMyBP-C was subsequently reported in mouse heart samples from the deoxycorticosterone acetate-salt hypertensive mouse model and correlated with diastolic dysfunction developed in these animals (Lovelock et al., 2012; Jeong et al., 2013). Further experiments in *in vitro* S-glutathiolated isolated myofibrils and detergent extracted fiber bundles identified three S-glutathiolation sites in murine cMyBP-C (cysteines 479, 627 and 655; Fig. 1) and reported to increase myofilament Ca^{2+} sensitivity (Patel et al., 2013). However, under those experimental conditions it cannot be ascertained that S-glutathiolation of cMyBP-C or another myofilament protein is responsible for the observed effects. Experiments in cardiac myocytes with ablation of either the full-length cMyBP-C or mutation of the identified S-glutathiolation sites will be useful to rule

out the contribution of other S-glutathiolated proteins. S-nitrosylation was found as another reversible oxidative modification of cMyBP-C. Mouse hearts were perfused with the S-nitrosylating agent S-nitrosoglutathione and analyzed by SNO-resin-assisted capture technology coupled tandem mass spectrometry. This identified Cys1270 in cMyBP-C as the site of modification (Fig. 1). However, the functional role of S-nitrosylation at that site and whether this occurs *in vivo* has not been investigated yet (Kohr et al., 2011).

Carbonylation of cMyBP-C as an irreversible oxidative modification occurring during conditions of prolonged oxidative stress was detected after exposure of spontaneously hypertensive rats manipulated to develop breast cancer to the chemotherapeutic ROS-producing drug doxorubicin (Aryal et al., 2014). Carbonylation of cMyBP-C could be an important contributor to cardiac dysfunction observed during chemotherapy (Aryal et al., 2014).

An important future direction will be to decipher the individual role of these newcoming exotic PTMs of cMyBP-C and to establish their contribution in preventing or promoting the development of cardiovascular disease. In this regard, a systematic study of the PTM fingerprint of cMyBP-C under disease conditions, and in relation to the known disease-causing mutations of the *MYBPC3* gene, could provide valuable information to pave the way for new therapeutic avenues to combat heart disease.

5. *MYBPC3*-targeting therapy for inherited cardiomyopathy

Recombinant adeno-associated viruses (AAV) have gained major interest in the past decade as suitable tools for investigating the functional role of individual genes, but also due to their therapeutic potential *in vivo* with selective tissue-tropism and persistent expression of the transgene in post-mitotic tissues (Zacchigna et al., 2014). In combination with the recent development of strategies targeting the endogenous mutation, mutant pre-mRNA or mutant mRNA, it is now possible to envision prevention or cure of inherited cardiomyopathy resulting from *MYBPC3* mutations, particularly of infants with bi-allelic truncating mutations with generally lethal consequences within the first year of life, without alternative therapeutic treatment options except heart transplantation.

Several targeting approaches have been developed in the past decade (Hammond and Wood, 2011; Doudna and Charpentier, 2014). The most recent is genome editing to correct a mutation by CRISPR/Cas9 technology (for review see (Hsu et al., 2014)). Naturally existing as part of the prokaryotic immune system, the CRISPR/Cas9 system has been used for correction of mutations in the mammalian genome (Ran et al., 2013). By inducing nicks in the double-stranded DNA and providing a template DNA sequence, it is possible to repair mutations by homologous recombination. CRISPR/Cas9 genome editing strategy corrected the *Dmd* gene in germline and prevented muscular dystrophy in mice (Long et al., 2014). Furthermore, it modified the *Psk9* gene in mouse liver after adenovirus gene transfer *in vivo* (Ding et al., 2014). This approach has not yet been evaluated for *MYBPC3* mutations, but it could be used for each single or clustered mutation, and therefore applied preferentially for frequent founder *MYBPC3* mutations (Fig. 3, Table 1). The potential of this strategy is currently under investigation in human iPSC lines. However, before translation to a clinical setting, initial teething problems need to be resolved (efficiency, off-target, long-term implications…).

Other strategies targeting the mutant pre-mRNA by exon skipping and/or *trans*-splicing have been evaluated for *MYBPC3*. Exon skipping can be achieved using antisense oligonucleotide (AONs) masking exonic splicing enhancer sequences, which prevent binding of the splicing machinery and therefore result in exclusion of the exon from the mRNA (Woodley and Valcarcel, 2002; Goyenvalle et al., 2004). This approach can be applied when the resulting shorter, but in-frame translated protein maintains its function. Proof-of-concept of exon skipping was recently shown in *Mybpc3*-targeted knock-in mice (Gedicke-Hornung et al., 2013). AONs directed against exons 5 and 6 were inserted in small nuclear RNA under the control of the U7 promoter and were packaged in tandem in AAV serotype 9, which has a predominant cardiotropism in mice. They induced the removal (i.e. skipping) of exons 5 and 6 and therefore an in-frame deletion, allowing the expression of an alternatively spliced *Mybpc3* mRNA variant, already present at low level in wild-type mice. Systemic administration of AAV-based AONs to *Mybpc3*-targeted knock-in newborn mice prevented both systolic dysfunction and left ventricular hypertrophy, at least for the duration of the investigated period (Gedicke-Hornung et al., 2013). For the human *MYBPC3* gene, skipping of 6 single exons or 5 double exons with specific AONs would result in shortened in-frame cMyBP-Cs, allowing the preservation of the functionally important phosphorylation and protein interaction sites (Fig. 3). With this approach, about half of missense or exonic/intronic truncating mutations could be removed, including 35 mutations in exon 25.

Another strategy is the spliceosome-mediated RNA *trans*-splicing. Hereby, two independently transcribed molecules, the mutant pre-

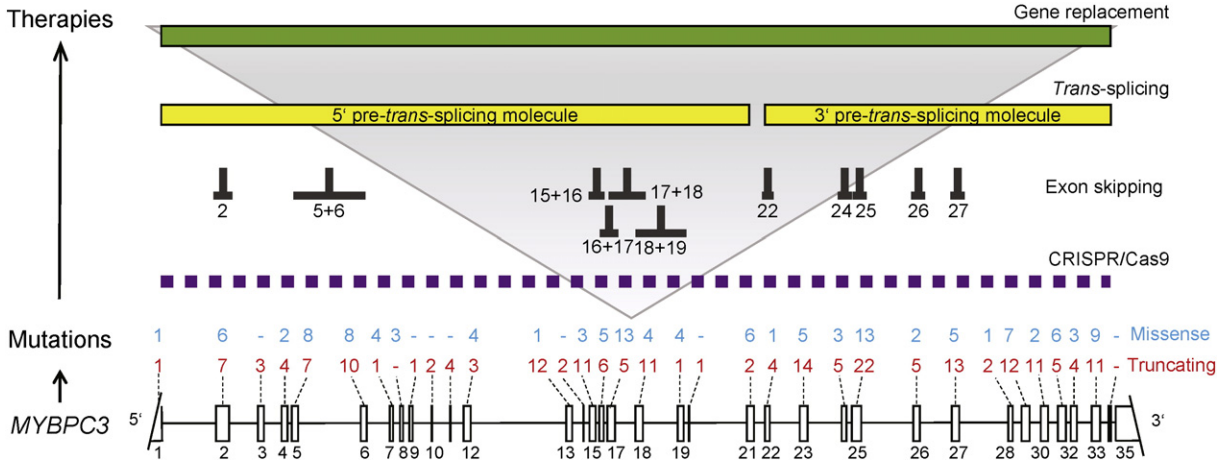

Fig. 3. Schematic representation of *MYBPC3* gene, mutations and options for causal therapy. *MYBPC3* encompasses 21 kbp and is composed of 35 exons. Exonic missense mutations (blue, total 129) and exonic and intronic truncating mutations (red, total 202) are shown on top of each exon. Causal therapies are shown as a reverse pyramide: i) CRISPR/Cas9 targeting single or a few mutations (purple dotted line), ii) exon skipping targeting all mutations present in one or two exons and associated introns (dark gray), iii) *trans*-splicing targeting either all 5′ mutations (exons 1 to 21; left yellow box) or 3′ mutations (exons 22 to 35; right yellow box), and finally iv) gene replacement targeting all mutations at once by gene transfer of the full-length *MYBPC3* cDNA (green box). Abbreviations used: CRISPR/Cas9, clustered regularly interspaced short palindromic repeat-associated system. A number of mutations are taken from (Behrens-Gawlik et al. 2014).

mRNA and the therapeutic pre-*trans*-splicing molecule carrying the wild-type sequence are spliced together to give rise to a repaired full-length mRNA (for review see Wally et al,. 2012). Recently, the feasibility of this method was shown both in isolated cardiac myocytes and in vivo in the heart of homozygous *Mybpc3*-targeted knock-in mice, although the efficiency of the process was low and the amount of repaired protein was not sufficient to prevent the development of the cardiac disease phenotype (Mearini et al., 2013). In principle, however, this strategy is superior to exon skipping or CRISPR/Cas9 genome editing and still attractive, because only two pre-*trans*-splicing molecules, targeting the 5′ and the 3′ of *MYBPC3* pre-mRNA would be sufficient to bypass all *MYBPC3* mutations associated with cardiomyopathies and therefore repair the mRNA (Fig. 3).

Finally, we recently showed that AAV-mediated gene transfer of the full-length *Mybpc3* (defined as "gene replacement", Fig. 3) dose-dependently prevents the development of cardiac hypertrophy and dysfunction over a 34-week period in homozygous *Mybpc3*-targeted knock-in mice (Mearini et al., 2014). Unexpectedly, the dose-dependent expression of exogenous *Mybpc3* was concomitantly associated with the down-regulation of endogenous mutant *Mybpc3*. Although the mechanism is not yet fully understood, and since the sarcomeric structure is tightly regulated, we believe that additional expression of a sarcomeric protein is expected to replace partially or completely the endogenous protein level in the sarcomere, as it has been shown in transgenic mice expressing sarcomeric proteins (for review, see Duncker et al., 2015). Therefore, this study paved the way for evaluating *MYBPC3* gene therapy in larger animal models, such as the naturally occurring cat model of HCM due to a *MYBPC3* mutation (Meurs et al., 2005), and to translate it to infants suffering from a fatal form of cardiomyopathy caused by *MYBPC3* bi-allelic mutations.

6. Outlook

MYBPC3 mutations are the most frequent mutations causing HCM and constitute a large part of other inherited cardiomyopathies such as DCM and LVNC. Founder HCM mutations have been identified in several countries and are associated with a higher risk to reveal homozygotes or compound heterozygotes, often associated with fatal forms of pediatric cardiomyopathy. For these forms in particular, there is a need for causal therapies. The recent findings that *Mybpc3* gene therapy can restore the level of cMyBP-C thus long-term preventing the disease phenotype in mice opens the way for testing this therapy in larger animal models with the vision to prevent heart failure development and decrease mortality in *MYBPC3*-associated pediatric cardiomyopathies.

Conflict of interest statement

The University Medical Center Hamburg-Eppendorf has filed a patent in the area of *MYBPC3* gene therapy using AAV9 for the treatment of hypertrophic cardiomyopathy. All authors have read the journal's policy on disclosure of potential conflict of interest.

Acknowledgments

This review and the corresponding Gene Wiki article are written as part of the Gene Wiki. Review series—a series resulting from a collaboration between the journal GENE and the Gene Wiki Initiative. The Gene Wiki Initiative is supported by the National Institutes of Health (GM089820). Additional support for Gene Wiki Reviews is provided by Elsevier, the publisher of GENE. This work was supported by the DZHK (German Center for Cardiovascular Research), the German Ministry of Research and Education (BMBF), the Association Institut de Myologie (Paris), a grant awarded to F. Cuello by the Deutsche Forschungsgemeinschaft (CU 53/2-1). The authors apologize that they have not been able to mention every important study in this field due to space constraints.

References

Ababou, A., Gautel, M., Pfuhl, M., 2007. Dissecting the N-terminal myosin binding site of human cardiac myosin-binding protein C. Structure and myosin binding of domain C2. J. Biol. Chem. 282, 9204–9215.

Adalsteinsdottir, B., Teekakirikul, P., Maron, B.J., Burke, M.A., Gudbjartsson, D.F., Holm, H., Stefansson, K., DePalma, S.R., Mazaika, E., McDonough, B., Danielsen, R., Seidman, J.G., Seidman, C.E., Gunnarsson, G.T., 2014. Nationwide study on hypertrophic cardiomyopathy in Iceland: evidence of a MYBPC3 founder mutation. Circulation 130, 1158–1167.

Aryal, B., Jeong, J., Rao, V.A., 2014. Doxorubicin-induced carbonylation and degradation of cardiac myosin binding protein C promote cardiotoxicity. Proc. Natl. Acad. Sci. U. S. A. 111, 2011–2016.

Authors/Task Force, m, Elliott, P.M., Anastasakis, A., Borger, M.A., Borggrefe, M., Cecchi, F., Charron, P., Hagege, A.A., Lafont, A., Limongelli, G., Mahrholdt, H., McKenna, W.J., Mogensen, J., Nihoyannopoulos, P., Nistri, S., Pieper, P.G., Pieske, B., Rapezzi, C., Rutten, F.H., Tillmanns, C., Watkins, H., 2014. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy: the task force for the diagnosis and management of hypertrophic cardiomyopathy of the European Society of Cardiology (ESC). Eur. Heart J. 35, 2733–2779.

Bahrudin, U., Morisaki, H., Morisaki, T., Ninomiya, H., Higaki, K., Nanba, E., Igawa, O., Takashima, S., Mizuta, E., Miake, J., Yamamoto, Y., Shirayoshi, Y., Kitakaze, M., Carrier, L., Hisatome, I., 2008. Ubiquitin-proteasome system impairment caused by a missense cardiac myosin-binding protein C mutation and associated with cardiac dysfunction in hypertrophic cardiomyopathy. J. Mol. Biol. 384, 896–907.

Bardswell, S.C., Cuello, F., Rowland, A.J., Sadayappan, S., Robbins, J., Gautel, M., Walker, J.W., Kentish, J.C., Avkiran, M., 2010. Distinct sarcomeric substrates are responsible for protein kinase D-mediated regulation of cardiac myofilament Ca^{2+} sensitivity and cross-bridge cycling. J. Biol. Chem. 285, 5674–5682.

Bardswell, S.C., Cuello, F., Kentish, J.C., Avkiran, M., 2012. cMyBP-C as a promiscuous substrate: phosphorylation by non-PKA kinases and its potential significance. J. Muscle Res. Cell Motil. 33, 53–60.

Barefield, D., Sadayappan, S., 2010. Phosphorylation and function of cardiac myosin binding protein-C in health and disease. J. Mol. Cell. Cardiol. 48, 866–875.

Barefield, D., Kumar, M., Gorham, J., Seidman, J.G., Seidman, C.E., de Tombe, P.P., Sadayappan, S., 2015. Haploinsufficiency of MYBPC3 exacerbates the development of hypertrophic cardiomyopathy in heterozygous mice. J. Mol. Cell. Cardiol. 79, 234–243.

Behrens-Gawlik, V., Mearini, G., Gedicke-Hornung, C., Richard, P., Carrier, L., 2014. MYBPC3 in hypertrophic cardiomyopathy: from mutation identification to RNA-based correction. Pflugers Arch. 466, 215–223.

Bonne, G., Carrier, L., Bercovici, J., Cruaud, C., Richard, P., Hainque, B., Gautel, M., Labeit, S., James, M., Beckmann, J., Weissenbach, J., Vosberg, H.P., Fiszman, M., Komajda, M., Schwartz, K., 1995. Cardiac myosin binding protein-C gene splice acceptor site mutation is associated with familial hypertrophic cardiomyopathy. Nat. Genet. 11, 438–440.

Brennan, J.P., Bardswell, S.C., Burgoyne, J.R., Fuller, W., Schroder, E., Wait, R., Begum, S., Kentish, J.C., Eaton, P., 2006. Oxidant-induced activation of type I protein kinase A is mediated by RI subunit interprotein disulfide bond formation. J. Biol. Chem. 281, 21827–21836.

Brickson, S., Fitzsimons, D.P., Pereira, L., Hacker, T., Valdivia, H., Moss, R.L., 2007. In vivo left ventricular functional capacity is compromised in cMyBP-C null mice. Am. J. Physiol. Heart Circ. Physiol. 292, H1747–H1754.

Calore, C., De Bortoli, M., Romualdi, C., Lorenzon, A., Angelini, A., Basso, C., Thiene, G., Iliceto, S., Rampazzo, A., Melacini, P., 2015. A founder MYBPC3 mutation results in HCM with a high risk of sudden death after the fourth decade of life. J. Med. Genet. 52, 338–347.

Carrier, L., Hengstenberg, C., Beckmann, J.S., Guicheney, P., Dufour, C., Bercovici, J., Dausse, E., Berebbi-Bertrand, I., Wisnewsky, C., Pulvenis, D., Fetler, L., Vignal, A., Weissenbach, J., Hillaire, D., Feingold, J., Bouhour, J.B., Hagege, A., Desnos, M., Isnard, R., Dubourg, O., Komajda, M., Schwartz, K., 1993. Mapping of a novel gene for familial hypertrophic cardiomyopathy to chromosome 11. Nat. Genet. 4, 311–313.

Carrier, L., Bonne, G., Bahrend, E., Yu, B., Richard, P., Niel, F., Hainque, B., Cruaud, C., Gary, F., Labeit, S., Bouhour, J.B., Dubourg, O., Desnos, M., Hagege, A.A., Trent, R.J., Komajda, M., Fiszman, M., Schwartz, K., 1997. Organization and sequence of human cardiac myosin binding protein C gene (MYBPC3) and identification of mutations predicted to produce truncated proteins in familial hypertrophic cardiomyopathy. Circ. Res. 80, 427–434.

Carrier, L., Bonne, G., Schwartz, K., 1998. Cardiac myosin binding protein C and hypertrophic cardiomyopathy. Trends Cardiovasc. Med. 8, 151–156.

Carrier, L., Knoell, R., Vignier, N., Keller, D.I., Bausero, P., Prudhon, B., Isnard, R., Ambroisine, M.L., Fiszman, M., Ross Jr., J., Schwartz, K., Chien, K.R., 2004. Asymmetric septal hypertrophy in heterozygous cMyBP-C null mice. Cardiovasc. Res. 63, 293–304.

Carrier, L., Schlossarek, S., Willis, M.S., Eschenhagen, T., 2010. The ubiquitin-proteasome system and nonsense-mediated mRNA decay in hypertrophic cardiomyopathy. Cardiovasc. Res. 85, 330–338.

Cazorla, O., Szilagyi, S., Vignier, N., Salazar, G., Kramer, E., Vassort, G., Carrier, L., Lacampagne, A., 2006. Length and protein kinase A modulations of myocytes in cardiac myosin binding protein C-deficient mice. Cardiovasc. Res. 69, 370–380.

Chen, P.P., Patel, J.R., Rybakova, I.N., Walker, J.W., Moss, R.L., 2010. Protein kinase A-induced myofilament desensitization to Ca(2+) as a result of phosphorylation of cardiac myosin-binding protein C. J. Gen. Physiol. 136, 615–627.

Christiaans, I., Nannenberg, E.A., Dooijes, D., Jongbloed, R.J., Michels, M., Postema, P.G., Majoor-Krakauer, D., van den Wijngaard, A., Mannens, M.M., van Tintelen, J.P., van Langen, I.M., Wilde, A.A., 2010. Founder mutations in hypertrophic cardiomyopathy patients in The Netherlands. Neth. Hear. J. 18, 248–254.

Copeland, O., Sadayappan, S., Messer, A.E., Steinen, G.J., van der Velden, J., Marston, S.B., 2010. Analysis of cardiac myosin binding protein-C phosphorylation in human heart muscle. J. Mol. Cell. Cardiol. 49, 1003–1011.
Cuello, F., Bardswell, S.C., Haworth, R.S., Ehler, E., Sadayappan, S., Kentish, J.C., Avkiran, M., 2011. Novel role for p90 ribosomal S6 kinase in the regulation of cardiac myofilament phosphorylation. J. Biol. Chem. 286, 5300–5310.
Dalle-Donne, I., Milzani, A., Gagliano, N., Colombo, R., Giustarini, D., Rossi, R., 2008. Molecular mechanisms and potential clinical significance of S-glutathionylation. Antioxid. Redox Signal. 10, 445–473.
Dellefave, L.M., Pytel, P., Mewborn, S., Mora, B., Guris, D.L., Fedson, S., Waggoner, D., Moskowitz, I., McNally, E.M., 2009. Sarcomere mutations in cardiomyopathy with left ventricular hypertrabeculation. Circ. Cardiovasc. Genet. 2, 442–449.
Dhandapany, P.S., Sadayappan, S., Xue, Y., Powell, G.T., Rani, D.S., Nallari, P., Rai, T.S., Khullar, M., Soares, P., Bahl, A., Tharkan, J.M., Vaideeswar, P., Rathinavel, A., Narasimhan, C., Ayapati, D.R., Ayub, Q., Mehdi, S.Q., Oppenheimer, S., Richards, M.B., Price, A.L., Patterson, N., Reich, D., Singh, L., Tyler-Smith, C., Thangaraj, K., 2009. A common MYBPC3 (cardiac myosin binding protein C) variant associated with cardiomyopathies in South Asia. Nat. Genet. 41, 187–191.
Ding, Q., Strong, A., Patel, K.M., Ng, S.L., Gosis, B.S., Regan, S.N., Cowan, C.A., Rader, D.J., Musunuru, K., 2014. Permanent alteration of PCSK9 with in vivo CRISPR-Cas9 genome editing. Circ. Res. 115, 488–492.
Dirkx, E., Cazorla, O., Schwenk, R.W., Lorenzen-Schmidt, I., Sadayappan, S., Van Lint, J., Carrier, L., van Eys, G.J., Glatz, J.F., Luiken, J.J., 2012. Protein kinase D increases maximal Ca^{2+}-activated tension of cardiomyocyte contraction by phosphorylation of cMyBP-C-Ser315. Am. J. Physiol. Heart Circ. Physiol. 303, H323–H331.
Doudna, J.A., Charpentier, E., 2014. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 346, 1258096.
Duncker, D.J., Bakkers, J., Brundel, B.J., Robbins, J., Tardiff, J.C., Carrier, L., 2015. Animal- and in silico models for the study of sarcomeric cardiomyopathies. Cardiovasc. Res.
El-Armouche, A., Pohlmann, L., Schlossarek, S., Starbatty, J., Yeh, Y.H., Nattel, S., Dobrev, D., Eschenhagen, T., Carrier, L., 2007. Decreased phosphorylation levels of cardiac myosin-binding protein-C in human and experimental heart failure. J. Mol. Cell. Cardiol. 43, 223–229.
Eschenhagen, T., Mummery, C., Knollmann, B.C., 2015. Modelling sarcomeric cardiomyopathies in the dish: from human heart samples to iPSC cardiomyocytes. Cardiovasc. Res. 105, 424–438.
Fert-Bober, J., Sokolove, J., 2014. Proteomics of citrullination in cardiovascular disease. Proteomics Clin. Appl. 8, 522–533.
Flashman, E., Redwood, C., Moolman-Smook, J., Watkins, H., 2004. Cardiac myosin binding protein C: its role in physiology and disease. Circ. Res. 94, 1279–1289.
Fougerousse, F., Delezoide, A.L., Fiszman, M.Y., Schwartz, K., Beckmann, J.S., Carrier, L., 1998. Cardiac myosin binding protein C gene is specifically expressed in heart during murine and human development. Circ. Res. 82, 130–133.
Fraysse, B., Weinberger, F., Bardswell, S.C., Cuello, F., Vignier, N., Geertz, B., Starbatty, J., Kramer, E., Coirault, C., Eschenhagen, T., Kentish, J.C., Avkiran, M., Carrier, L., 2012. Increased myofilament Ca^{2+} sensitivity and diastolic dysfunction as early consequences of Mybpc3 mutation in heterozygous knock-in mice. J. Mol. Cell. Cardiol. 52, 1299–1307.
Garcia-Castro, M., Reguero, J.R., Alvarez, V., Batalla, A., Soto, M.I., Albaladejo, V., Coto, E., 2005. Hypertrophic cardiomyopathy linked to homozygosity for a new mutation in the myosin-binding protein C gene (A627V) suggests a dosage effect. Int. J. Cardiol. 102, 501–507.
Gautel, M., Zuffardi, O., Freiburg, A., Labeit, S., 1995. Phosphorylation switches specific for the cardiac isoform of myosin binding protein-C: a modulator of cardiac contraction? Embo J. 14, 1952–1960.
Ge, Y., Rybakova, I.N., Xu, Q., Moss, R.L., 2009. Top-down high-resolution mass spectrometry of cardiac myosin binding protein C revealed that truncation alters protein phosphorylation state. Proc. Natl. Acad. Sci. U. S. A. 106, 12658–12663.
Gedicke-Hornung, C., Behrens-Gawlik, V., Reischmann, S., Geertz, B., Stimpel, D., Weinberger, F., Schlossarek, S., Precigout, G., Braren, I., Eschenhagen, T., Mearini, G., Lorain, S., Voit, T., Dreyfus, P.A., Garcia, L., Carrier, L., 2013. Rescue of cardiomyopathy through U7snRNA-mediated exon skipping in Mybpc3-targeted knock-in mice. EMBO Mol. Med. 5 1128–45.
Geisterfer-Lowrance, A.A., Kass, S., Tanigawa, G., Vosberg, H.P., McKenna, W., Seidman, C.E., Seidman, J.G., 1990. A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin-heavy chain gene missense mutation. Cell 62, 999–1006.
Girolami, F., Olivotto, I., Passerini, I., Zachara, E., Nistri, S., Re, F., Fantini, S., Baldini, K., Torricelli, F., Cecchi, F., 2006. A molecular screening strategy based on beta-myosin heavy chain, cardiac myosin binding protein C and troponin T genes in Italian patients with hypertrophic cardiomyopathy. J. Cardiovasc. Med. (Hagerstown) 7 601–7.
Govindan, S., Sarkey, J., Ji, X., Sundaresan, N.R., Gupta, M.P., de Tombe, P.P., Sadayappan, S., 2012. Pathogenic properties of the N-terminal region of cardiac myosin binding protein-C in vitro. J. Muscle Res. Cell Motil. 33, 17–30.
Goyenvalle, A., Vulin, A., Fougerousse, F., Leturcq, F., Kaplan, J.C., Garcia, L., Danos, O., 2004. Rescue of dystrophic muscle through U7 snRNA-mediated exon skipping. Science 306 1796–9.
Gruen, M., Gautel, M., 1999. Mutations in beta-myosin S2 that cause familial hypertrophic cardiomyopathy (FHC) abolish the interaction with the regulatory domain of myosin binding protein-C. J. Mol. Biol. 286, 933–949.
Gupta, M.K., Robbins, J., 2014. Post-translational control of cardiac hemodynamics through myosin binding protein C. Pflugers Arch. 466, 231–236.
Haas, J., Frese, K.S., Peil, B., Kloos, W., Keller, A., Nietsch, R., Feng, Z., Muller, S., Kayvanpour, E., Vogel, B., Sedaghat-Hamedani, F., Lim, W.K., Zhao, X., Fradkin, D., Kohler, D., Fischer, S., Franke, J., Marquart, S., Barb, I., Li, D.T., Amr, A., Ehlermann, P., Mereles, D., Weis, T., Hassel, S., Kremer, A., King, V., Wirsz, E., Isnard, R., Komajda, M., Serio, A., Grasso, M., Syrris, P., Wicks, E., Plagnol, V., Lopes, L., Gadgaard, T., Eiskjaer, H., Jorgensen, M., Garcia-Giustiniani, D., Ortiz-Genga, M., Crespo-Leiro, M.G., Deprez, R.H., Christiaans, I., van Rijsingen, I.A., Wilde, A.A., Waldenstrom, A., Bolognesi, M., Bellazzi, R., Morner, S., Bermejo, J.L., Monserrat, L., Villard, E., Mogensen, J., Pinto, Y.M., Charron, P., Elliott, P., Arbustini, E., Katus, H.A., Meder, B., 2014. Atlas of the clinical genetics of human dilated cardiomyopathy. Eur. Heart J.
Hammond, S.M., Wood, M.J., 2011. Genetic therapies for RNA mis-splicing diseases. Trends Genet. 27, 196–205.
Han, L., Li, Y., Tchao, J., Kaplan, A.D., Lin, B., Li, Y., Mich-Basso, J., Lis, A., Hassan, N., London, B., Bett, G.C., Tobita, K., Rasmusson, R.L., Yang, L., 2014. Study familial hypertrophic cardiomyopathy using patient-specific induced pluripotent stem cells. Cardiovasc. Res. 104, 258–269.
Harris, S.P., Bartley, C.R., Hacker, T.A., McDonald, K.S., Douglas, P.S., Greaser, M.L., Powers, P.A., Moss, R.L., 2002. Hypertrophic cardiomyopathy in cardiac myosin binding protein-C knockout mice. Circ. Res. 90, 594–601.
Harris, S.P., Rostkova, E., Gautel, M., Moss, R.L., 2004. Binding of myosin binding protein-C to myosin subfragment S2 affects contractility independent of a tether mechanism. Circ. Res. 95, 930–936.
Hartzell, H.C., Glass, D.B., 1984. Phosphorylation of purified cardiac muscle C-protein by purified cAMP-dependent and endogenous Ca^{2+}-calmodulin-dependent protein kinases. J. Biol. Chem. 259, 15587–15596.
Hartzell, H.C., Titus, L., 1982. Effects of cholinergic and adrenergic agonists on phosphorylation of a 165,000-Dalton myofibrillar protein in intact cardiac muscle. J. Biol. Chem. 257, 2111–2120.
Haworth, R.S., Cuello, F., Herron, T.J., Franzen, G., Kentish, J.C., Gautel, M., Avkiran, M., 2004. Protein kinase D is a novel mediator of cardiac troponin I phosphorylation and regulates myofilament function. Circ. Res. 95, 1091–1099.
Ho, C.Y., Carlsen, C., Thune, J.J., Havndrup, O., Bundgaard, H., Farrohi, F., Rivero, J., Cirino, A.L., Andersen, P.S., Christiansen, M., Maron, B.J., Orav, E.J., Kober, L., 2009. Echocardiographic strain imaging to assess early and late consequences of sarcomere mutations in hypertrophic cardiomyopathy. Circ. Cardiovasc. Genet. 2, 314–321.
Ho, C.Y., Charron, P., Richard, P., Girolami, F., Van Spaendonck-Zwarts, K.Y., Pinto, Y., 2015. Genetic advances in sarcomeric cardiomyopathies: state of the art. Cardiovasc. Res. 105, 397–408.
Hoedemaekers, Y.M., Caliskan, K., Michels, M., Frohn-Mulder, I., van der Smagt, J.J., Phefferkorn, J.E., Wessels, M.W., ten Cate, F.J., Sijbrands, E.J., Dooijes, D., Majoor-Krakauer, D.F., 2010. The importance of genetic counseling, DNA diagnostics, and cardiologic family screening in left ventricular noncompaction cardiomyopathy. Circ. Cardiovasc. Genet. 3, 232–239.
Hofmann, P.A., Hartzell, H.C., Moss, R.L., 1991. Alterations in Ca^{2+} sensitive tension due to partial extraction of C-protein from rat skinned cardiac myocytes and rabbit skeletal muscle fibers. J. Gen. Physiol. 97, 1141–1163.
Hsu, P.D., Lander, E.S., Zhang, F., 2014. Development and applications of CRISPR-Cas9 for genome engineering. Cell 157, 1262–1278.
Ingles, J., Doolan, A., Chiu, C., Seidman, J., Seidman, C., Semsarian, C., 2005. Compound and double mutations in patients with hypertrophic cardiomyopathy: implications for genetic testing and counselling. J. Med. Genet. 42, e59.
Jaaskelainen, P., Miettinen, R., Karkkainen, P., Toivonen, L., Laakso, M., Kuusisto, J., 2004. Genetics of hypertrophic cardiomyopathy in eastern Finland: few founder mutations with benign or intermediary phenotypes. Ann. Med. 36, 23–32.
Jeong, E.M., Monasky, M.M., Gu, L., Taglieri, D.M., Patel, B.G., Liu, H., Wang, Q., Greener, I., Dudley Jr., S.C., Solaro, R.J., 2013. Tetrahydrobiopterin improves diastolic dysfunction by reversing changes in myofilament properties. J. Mol. Cell. Cardiol. 56, 44–54.
Kampourakis, T., Yan, Z., Gautel, M., Sun, Y.B., Irving, M., 2014. Myosin binding protein-C activates thin filaments and inhibits thick filaments in heart muscle cells. Proc. Natl. Acad. Sci. U. S. A. 111, 18763–18768.
Kohr, M.J., Aponte, A.M., Sun, J., Wang, G., Murphy, E., Gucek, M., Steenbergen, C., 2011. Characterization of potential S-nitrosylation sites in the myocardium. Am. J. Physiol. Heart Circ. Physiol. 300, H1327–H1335.
Korte, F.S., McDonald, K.S., Harris, S.P., Moss, R.L., 2003. Loaded shortening, power output, and rate of force redevelopment are increased with knockout of cardiac myosin binding protein-C. Circ. Res. 93, 752–758.
Kubo, T., Kitaoka, H., Okawa, M., Matsumura, Y., Hitomi, N., Yamasaki, N., Furuno, T., Takata, J., Nishinaga, M., Kimura, A., Doi, Y.L., 2005. Lifelong left ventricular remodeling of hypertrophic cardiomyopathy caused by a founder frameshift deletion mutation in the cardiac myosin-binding protein C gene among Japanese. J. Am. Coll. Cardiol. 46, 1737–1743.
Kulikovskaya, I., McClellan, G., Flavigny, J., Carrier, L., Winegrad, S., 2003. Effect of MyBP-C binding to actin on contractility in heart muscle. J. Gen. Physiol. 122, 1–15.
Kunst, G., Kress, K.R., Gruen, M., Uttenweiler, D., Gautel, M., Fink, R.H., 2000. Myosin binding protein C, a phosphorylation-dependent force regulator in muscle that controls the attachment of myosin heads by its interaction with myosin S2. Circ. Res. 86, 51–58.
Kuster, D.W., Sequeira, V., Najafi, A., Boontje, N.M., Wijnker, P.J., Witjas-Paalberends, E.R., Marston, S.B., Dos Remedios, C.G., Carrier, L., Demmers, J.A., Redwood, C., Sadayappan, S., van der Velden, J., 2013. GSK3beta phosphorylates newly identified site in the proline–alanine-rich region of cardiac myosin-binding protein C and alters cross-bridge cycling kinetics in human: short communication. Circ. Res. 112, 633–639.
Lan, F., Lee, A.S., Liang, P., Sanchez-Freire, V., Nguyen, P.K., Wang, L., Han, L., Yen, M., Wang, Y., Sun, N., Abilez, O.J., Hu, S., Ebert, A.D., Navarrete, E.G., Simmons, C.S., Wheeler, M., Pruitt, B., Lewis, R., Yamaguchi, Y., Ashley, E.A., Bers, D.M., Robbins, R.C., Longaker, M.T., Wu, J.C., 2013. Abnormal calcium handling properties underlie familial hypertrophic cardiomyopathy pathology in patient-specific induced pluripotent stem cells. Cell Stem Cell 12, 101–113.
Lekanne Deprez, R.H., Muurling-Vlietman, J.J., Hruda, J., Baars, M.J., Wijnaendts, L.C., Stolte-Dijkstra, I., Alders, M., van Hagen, J.M., 2006. Two cases of severe neonatal hypertrophic cardiomyopathy caused by compound heterozygous mutations in the MYBPC3 gene. J. Med. Genet. 43, 829–832.

Long, C., McAnally, J.R., Shelton, J.M., Mireault, A.A., Bassel-Duby, R., Olson, E.N., 2014. Prevention of muscular dystrophy in mice by CRISPR/Cas9-mediated editing of germline DNA. Science 345, 1184–1188.
Lovelock, J.D., Monasky, M.M., Jeong, E.M., Lardin, H.A., Liu, H., Patel, B.G., Taglieri, D.M., Gu, L., Kumar, P., Pokhrel, N., Zeng, D., Belardinelli, L., Sorescu, D., Solaro, R.J., Dudley Jr., S.C., 2012. Ranolazine improves cardiac diastolic dysfunction through modulation of myofilament calcium sensitivity. Circ. Res. 110, 841–850.
Luther, P.K., Bennett, P.M., Knupp, C., Craig, R., Padron, R., Harris, S.P., Patel, J., Moss, R.L., 2008. Understanding the organisation and role of myosin binding protein C in normal striated muscle by comparison with MyBP-C knockout cardiac muscle. J. Mol. Biol. 384, 60–72.
Luther, P.K., Winkler, H., Taylor, K., Zoghbi, M.E., Craig, R., Padron, R., Squire, J.M., Liu, J., 2011. Direct visualization of myosin-binding protein C bridging myosin and actin filaments in intact muscle. Proc. Natl. Acad. Sci. U. S. A. 108, 11423–11428.
Maron, B.J., Gardin, J.M., Flack, J.M., Gidding, S.S., Kurosaki, T.T., Bild, D.E., 1995. Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA Study. Coronary artery risk development in (young) adults. Circulation 92, 785–789.
Maron, B.J., Maron, M.S., Semsarian, C., 2012. Double or compound sarcomere mutations in hypertrophic cardiomyopathy: a potential link to sudden death in the absence of conventional risk factors. Heart Rhythm. 9, 57–63.
Marston, S., Copeland, O.N., Jacques, A., Livesey, K., Tsang, V., McKenna, W.J., Jalilzadeh, S., Carballo, S., Redwood, C., Watkins, H., 2009. Evidence from human myectomy samples that MYBPC3 mutations cause hypertrophic cardiomyopathy through haploinsufficiency. Circ. Res. 105, 219–222.
Marston, S., Copeland, O., Gehmlich, K., Schlossarek, S., Carrier, L., 2012. How do MYBPC3 mutations cause hypertrophic cardiomyopathy? J. Muscle Res. Cell Motil. 33, 75–80.
Marziliano, N., Merlini, P.A., Vignati, G., Orsini, F., Motta, V., Bandiera, L., Intrieri, M., Veronese, S., 2012. A case of compound mutations in the MYBPC3 gene associated with biventricular hypertrophy and neonatal death. Neonatology 102, 254–258.
McClellan, G., Kulikovskaya, I., Winegrad, S., 2001. Changes in cardiac contractility related to calcium-mediated changes in phosphorylation of myosin-binding protein C. Biophys. J. 81, 1083–1092.
Mearini, G., Schlossarek, S., Willis, M.S., Carrier, L., 2008. The ubiquitin–proteasome system in cardiac dysfunction. Biochim. Biophys. Acta 1782, 749–763.
Mearini, G., Stimpel, D., Kramer, E., Geertz, B., Braren, I., Gedicke-Hornung, C., Precigout, G., Muller, O.J., Katus, H.A., Eschenhagen, T., Voit, T., Garcia, L., Lorain, S., Carrier, L., 2013. Repair of Mybpc3 mRNA by 5′trans-splicing in a mouse model of hypertrophic cardiomyopathy. Mol. Ther. Nucleic Acids 2, e102.
Mearini, G., Stimpel, D., Geertz, B., Weinberger, F., Krämer, E., Schlossarek, S., Mourot-Filiatre, J., Stöhr, A., Dutshc, A., Wijnker, P.J.M., Braren, I., Katus, H.A., Müller, O.J., Voit, T., Eschenhagen, T., Carrier, L., 2014. *Mybpc3* gene therapy for neonatal cardiomyopathy enables longterm disease prevention in mice. Nat. Commun. 5, 5515.
Meurs, K.M., Sanchez, X., David, R.M., Bowles, N.E., Towbin, J.A., Reiser, P.J., Kittleson, J.A., Munro, M.J., Dryburgh, K., Macdonald, K.A., Kittleson, M.D., 2005. A cardiac myosin binding protein C mutation in the Maine Coon cat with familial hypertrophic cardiomyopathy. Hum. Mol. Genet. 14, 3587–3593.
Michels, M., Soliman, O.I., Kofflard, M.J., Hoedemaekers, Y.M., Dooijes, D., Majoor-Krakauer, D., ten Cate, F.J., 2009. Diastolic abnormalities as the first feature of hypertrophic cardiomyopathy in Dutch myosin-binding protein C founder mutations. JACC Cardiovasc. Imaging 2, 58–64.
Mohamed, A.S., Dignam, J.D., Schlender, K.K., 1998. Cardiac myosin-binding protein C (MyBP-C): identification of protein kinase A and protein kinase C phosphorylation sites. Arch. Biochem. Biophys. 358, 313–319.
Moolman, J.A., Reith, S., Uhl, K., Bailey, S., Gautel, M., Jeschke, B., Fischer, C., Ochs, J., McKenna, W.J., Klues, H., Vosberg, H.P., 2000. A newly created splice donor site in exon 25 of the MyBP-C gene is responsible for inherited hypertrophic cardiomyopathy with incomplete disease penetrance. Circulation 101, 1396–1402.
Morita, H., Rehm, H.L., Menesses, A., McDonough, B., Roberts, A.E., Kucherlapati, R., Towbin, J.A., Seidman, J.G., Seidman, C.E., 2008. Shared genetic causes of cardiac hypertrophy in children and adults. N. Engl. J. Med. 358, 1899–1908.
Moss, R.L., Fitzsimons, D.P., Ralphe, J.C., 2015. Cardiac MyBP-C regulates the rate and force of contraction in mammalian myocardium. Circ. Res. 116, 183–192.
Mun, J.Y., Gulick, J., Robbins, J., Woodhead, J., Lehman, W., Craig, R., 2011. Electron microscopy and 3D reconstruction of F-actin decorated with cardiac myosin-binding protein C (cMyBP-C). J. Mol. Biol. 410, 214–225.
Mun, J.Y., Previs, M.J., Yu, H.Y., Gulick, J., Tobacman, L.S., Beck Previs, S., Robbins, J., Warshaw, D.M., Craig, R., 2014. Myosin-binding protein C displaces tropomyosin to activate cardiac thin filaments and governs their speed by an independent mechanism. Proc. Natl. Acad. Sci. U. S. A. 111, 2170–2175.
Nanni, L., Pieroni, M., Chimenti, C., Simionati, B., Zimbello, R., Maseri, A., Frustaci, A., Lanfranchi, G., 2003. Hypertrophic cardiomyopathy: two homozygous cases with "typical" hypertrophic cardiomyopathy and three new mutations in cases with progression to dilated cardiomyopathy. Biochem. Biophys. Res. Commun. 309, 391–398.
Niimura, H., Bachinski, L.L., Sangwatanaroj, S., Watkins, H., Chudley, A.E., McKenna, W., Kristinsson, A., Roberts, R., Sole, M., Maron, B.J., Seidman, J.G., Seidman, C.E., 1998. Mutations in the gene for cardiac myosin-binding protein C and late-onset familial hypertrophic cardiomyopathy. N. Engl. J. Med. 338, 1248–1257.
Olivotto, I., d'Amati, G., Basso, C., Van Rossum, A., Patten, M., Emdin, M., Pinto, Y., Tomberli, B., Camici, P.G., Michels, M., 2015. Defining phenotypes and disease progression in sarcomeric cardiomyopathies: contemporary role of clinical investigations. Cardiovasc. Res. 105, 409–423.
Ortiz, M.F., Rodriguez-Garcia, M.I., Hermida-Prieto, M., Fernandez, X., Veira, E., Barriales-Villa, R., Castro-Beiras, A., Monserrat, L., 2009. A homozygous MYBPC3 gene mutation associated with a severe phenotype and a high risk of sudden death in a family with hypertrophic cardiomyopathy. Rev. Esp. Cardiol. 62, 572–575.
Otsuka, H., Arimura, T., Abe, T., Kawai, H., Aizawa, Y., Kubo, T., Kitaoka, H., Nakamura, H., Nakamura, K., Okamoto, H., Ichida, F., Ayusawa, M., Nunoda, S., Isobe, M., Matsuzaki, M., Doi, Y.L., Fukuda, K., Sasaoka, T., Izumi, T., Ashizawa, N., Kimura, A., 2012. Prevalence and distribution of sarcomeric gene mutations in Japanese patients with familial hypertrophic cardiomyopathy. Circ. J. 76, 453–461.
Patel, B.G., Wilder, T., Solaro, R.J., 2013. Novel control of cardiac myofilament response to calcium by S-glutathionylation at specific sites of myosin binding protein C. Front. Physiol. 4, 336.
Pfuhl, M., Gautel, M., 2012. Structure, interactions and function of the N-terminus of cardiac myosin binding protein C (MyBP-C): who does what, with what, and to whom? J. Muscle Res. Cell Motil. 33, 83–94.
Pohlmann, L., Kroger, I., Vignier, N., Schlossarek, S., Kramer, E., Coirault, C., Sultan, K.R., El-Armouche, A., Winegrad, S., Eschenhagen, T., Carrier, L., 2007. Cardiac myosin-binding protein C is required for complete relaxation in intact myocytes. Circ. Res. 101, 928–938.
Predmore, J.M., Wang, P., Davis, F., Bartolone, S., Westfall, M.V., Dyke, D.B., Pagani, F., Powell, S.R., Day, S.M., 2010. Ubiquitin proteasome dysfunction in human hypertrophic and dilated cardiomyopathies. Circulation 121, 997–1004.
Previs, M.J., Prosser, B.L., Mun, J.Y., Previs, S.B., Gulick, J., Lee, K., Robbins, J., Craig, R., Lederer, W.J., Warshaw, D.M., 2015. Myosin-binding protein C corrects an intrinsic inhomogeneity in cardiac excitation–contraction coupling. Sci. Adv. 1.
Probst, S., Oechslin, E., Schuler, P., Greutmann, M., Boye, P., Knirsch, W., Berger, F., Thierfelder, L., Jenni, R., Klaassen, S., 2011. Sarcomere gene mutations in isolated left ventricular noncompaction cardiomyopathy do not predict clinical phenotype. Circ. Cardiovasc. Genet. 4, 367–374.
Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A., Zhang, F., 2013. Genome engineering using the CRISPR-Cas9 system. Nat. Protoc. 8, 2281–2308.
Razumova, M.V., Shaffer, J.F., Tu, A.Y., Flint, G.V., Regnier, M., Harris, S.P., 2006. Effects of the N-terminal domains of myosin binding protein-C in an in vitro motility assay: evidence for long-lived cross-bridges. J. Biol. Chem. 281, 35846–35854.
Richard, P., Charron, P., Carrier, L., Ledeuil, C., Cheav, T., Pichereau, C., Benaiche, A., Isnard, R., Dubourg, O., Burban, M., Gueffet, J.P., Millaire, A., Desnos, M., Schwartz, K., Hainque, B., Komajda, M., 2003. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations and implications for molecular diagnosis strategy. Circulation 107, 2227–2232.
Rottbauer, W., Gautel, M., Zehelein, J., Labeit, S., Franz, W.M., Fischer, C., Vollrath, B., Mall, G., Dietz, R., Kubler, W., Katus, H.A., 1997. Novel splice donor site mutation in the cardiac myosin-binding protein-C gene in familial hypertrophic cardiomyopathy. Characterization of cardiac transcript and protein. J. Clin. Invest. 100, 475–482.
Rybakova, I.N., Greaser, M.L., Moss, R.L., 2011. Myosin binding protein C interaction with actin: characterization and mapping of the binding site. J. Biol. Chem. 286, 2008–2016.
Sadayappan, S., de Tombe, P.P., 2012. Cardiac myosin binding protein-C: redefining its structure and function. Biophys. Rev. 4, 93–106.
Sadayappan, S., Gulick, J., Osinska, H., Martin, L.A., Hahn, H.S., Dorn 2nd, G.W., Klevitsky, R., Seidman, C.E., Seidman, J.G., Robbins, J., 2005. Cardiac myosin-binding protein-C phosphorylation and cardiac function. Circ. Res. 97, 1156–1163.
Sadayappan, S., Osinska, H., Klevitsky, R., Lorenz, J.N., Sargent, M., Molkentin, J.D., Seidman, C.E., Seidman, J.G., Robbins, J., 2006. Cardiac myosin binding protein c phosphorylation is cardioprotective. Proc. Natl. Acad. Sci. U. S. A. 103, 16918–16923.
Sadayappan, S., Gulick, J., Osinska, H., Barefield, D., Cuello, F., Avkiran, M., Lasko, V.M., Lorenz, J.N., Maillet, M., Martin, J.L., Brown, J.H., Bers, D.M., Molkentin, J.D., James, J., Robbins, J., 2011. A critical function for ser-282 in cardiac myosin binding protein-C phosphorylation and cardiac function. Circ. Res. 109, 141–150.
Saltzman, A.J., Mancini-DiNardo, D., Li, C., Chung, W.K., Ho, C.Y., Hurst, S., Wynn, J., Care, M., Hamilton, R.M., Seidman, G.W., Gorham, J., McDonough, B., Sparks, E., Seidman, J.G., Seidman, C.E., Rehm, H.L., 2010. Short communication: the cardiac myosin binding protein C Arg502Trp mutation: a common cause of hypertrophic cardiomyopathy. Circ. Res. 106, 1549–1552.
Sarikas, A., Carrier, L., Schenke, C., Doll, D., Flavigny, J., Lindenberg, K.S., Eschenhagen, T., Zolk, O., 2005. Impairment of the ubiquitin-proteasome system by truncated cardiac myosin binding protein C mutants. Cardiovasc. Res. 66, 33–44.
Schaefer, E., Helms, P., Marcellin, L., Desprez, P., Billaud, P., Chanavat, V., Rousson, R., Millat, G., 2014. Next-generation sequencing (NGS) as a fast molecular diagnosis tool for left ventricular noncompaction in an infant with compound mutations in the MYBPC3 gene. Eur. J. Med. Genet. 57, 129–132.
Schlossarek, S., Mearini, G., Carrier, L., 2011. Cardiac myosin-binding protein C in hypertrophic cardiomyopathy: mechanisms and therapeutic opportunities. J. Mol. Cell. Cardiol. 50, 613–620.
Schlossarek, S., Englmann, D.R., Sultan, K.R., Sauer, M., Eschenhagen, T., Carrier, L., 2012a. Defective proteolytic systems in Mybpc3-targeted mice with cardiac hypertrophy. Basic Res. Cardiol. 107, 1–13.
Schlossarek, S., Schuermann, F., Geertz, B., Mearini, G., Eschenhagen, T., Carrier, L., 2012b. Adrenergic stress reveals septal hypertrophy and proteasome impairment in heterozygous Mybpc3-targeted knock-in mice. J. Muscle Res. Cell Motil. 33, 5–15.
Schlossarek, S., Frey, N., Carrier, L., 2014. Ubiquitin–proteasome system and hereditary cardiomyopathies. J. Mol. Cell. Cardiol. 71, 25–31.
Sequeira, V., Wijnker, P.J., Nijenkamp, L.L., Kuster, D.W., Najafi, A., Witjas-Paalberends, E.R., Regan, J.A., Boontje, N., Ten Cate, F.J., Germans, T., Carrier, L., Sadayappan, S., van Slegtenhorst, M.A., Zaremba, R., Foster, D.B., Murphy, A.M., Poggesi, C., Dos Remedios, C., Stienen, G.J., Ho, C.Y., Michels, M., van der Velden, J., 2013. Perturbed length-dependent activation in human hypertrophic cardiomyopathy with missense sarcomeric gene mutations. Circ. Res. 112, 1491–1505.
Stelzer, J.E., Dunning, S.B., Moss, R.L., 2006a. Ablation of cardiac myosin-binding protein-C accelerates stretch activation in murine skinned myocardium. Circ. Res. 98, 1212–1218.
Stelzer, J.E., Fitzsimons, D.P., Moss, R.L., 2006b. Ablation of myosin-binding protein-C accelerates force development in mouse myocardium. Biophys. J. 90, 4119–4127.

Stohr, A., Friedrich, F.W., Flenner, F., Geertz, B., Eder, A., Schaaf, S., Hirt, M.N., Uebeler, J., Schlossarek, S., Carrier, L., Hansen, A., Eschenhagen, T., 2013. Contractile abnormalities and altered drug response in engineered heart tissue from Mybpc3-targeted knock-in mice. J. Mol. Cell. Cardiol. 63, 189–198.

Sun, N., Yazawa, M., Liu, J., Han, L., Sanchez-Freire, V., Abilez, O.J., Navarrete, E.G., Hu, S., Wang, L., Lee, A., Pavlovic, A., Lin, S., Chen, R., Hajjar, R.J., Snyder, M.P., Dolmetsch, R.E., Butte, M.J., Ashley, E.A., Longaker, M.T., Robbins, R.C., Wu, J.C., 2012. Patient-specific induced pluripotent stem cells as a model for familial dilated cardiomyopathy. Sci. Transl. Med. 4, 130ra47.

Tajsharghi, H., Leren, T.P., Abdul-Hussein, S., Tulinius, M., Brunvand, L., Dahl, H.M., Oldfors, A., 2010. Unexpected myopathy associated with a mutation in MYBPC3 and misplacement of the cardiac myosin binding protein C. J. Med. Genet. 47, 575–577.

Tanaka, A., Yuasa, S., Mearini, G., Egashira, T., Seki, T., Kodaira, M., Kusumoto, D., Kuroda, Y., Okata, S., Suzuki, T., Inohara, T., Arimura, T., Makino, S., Kimura, K., Kimura, A., Furukawa, T., Carrier, L., Node, K., Fukuda, K., 2014. Endothelin-1 induces myofibrillar disarray and contractile vector variability in hypertrophic cardiomyopathy-induced pluripotent stem cell-derived cardiomyocytes. J. Am. Heart Assoc. 3, e001263.

Teirlinck, C.H., Senni, F., Malti, R.E., Majoor-Krakauer, D., Fellmann, F., Millat, G., Andre-Fouet, X., Pernot, F., Stumpf, M., Boutarin, J., Bouvagnet, P., 2012. A human MYBPC3 mutation appearing about 10 centuries ago results in a hypertrophic cardiomyopathy with delayed onset, moderate evolution but with a risk of sudden death. BMC Med. Genet. 13, 105.

Thierfelder, L., Watkins, H., MacRae, C., Lamas, R., McKenna, W., Vosberg, H.P., Seidman, J.G., Seidman, C.E., 1994. Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. Cell 77, 701–712.

Thottakara, T., Friedrich, F.W., Reischmann, S., Braumann, S., Schlossarek, S., Krämer, E., Juhr, D., Schlüter, H., 2015. The E3 ubiquitin ligase Asb2β is downregulated in a mouse model of hypertrophic cardiomyopathy and Targets desmin for proteasomal Degradation. J. Mol. Cell. Cardiol. available online: 3-SEP-2015.

van der Velden, J., Ho, C.Y., Tardiff, J.C., Olivotto, I., Knollmann, B.C., Carrier, L., 2015. Research priorities in sarcomeric cardiomyopathies. Cardiovasc. Res. 105, 449–456.

van Dijk, S.J., Dooijes, D., Dos Remedios, C., Michels, M., Lamers, J.M., Winegrad, S., Schlossarek, S., Carrier, L., Ten Cate, F.J., Stienen, G.J., van der Velden, J., 2009. Cardiac myosin-binding protein C mutations and hypertrophic cardiomyopathy. Haploinsufficiency, deranged phosphorylation, and cardiomyocyte dysfunction. Circulation 119, 1473–1483.

van Dijk, S.J., Paalberends, E.R., Najafi, A., Michels, M., Sadayappan, S., Carrier, L., Boontje, N.M., Kuster, D.W., van Slegtenhorst, M., Dooijes, D., Dos Remedios, C., Ten Cate, F.J., Stienen, G.J., van der Velden, J., 2012. Contractile dysfunction irrespective of the mutant protein in human hypertrophic cardiomyopathy with normal systolic function. Circ. Heart Fail. 5, 36–46.

Van Driest, S.L., Vasile, V.C., Ommen, S.R., Will, M.L., Tajik, A.J., Gersh, B.J., Ackerman, M.J., 2004. Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. J. Am. Coll. Cardiol. 44, 1903–1910.

Vignier, N., Schlossarek, S., Fraysse, B., Mearini, G., Kramer, E., Pointu, H., Mougenot, N., Guiard, J., Reimer, R., Hohenberg, H., Schwartz, K., Vernet, M., Eschenhagen, T., Carrier, L., 2009. Nonsense-mediated mRNA decay and ubiquitin–proteasome system regulate cardiac myosin-binding protein C mutant levels in cardiomyopathic mice. Circ. Res. 105, 239–248.

Waldmuller, S., Sakthivel, S., Saadi, A.V., Selignow, C., Rakesh, P.G., Golubenko, M., Joseph, P.K., Padmakumar, R., Richard, P., Schwartz, K., Tharakan, J.M., Rajamanickam, C., Vosberg, H.P., 2003. Novel deletions in MYH7 and MYBPC3 identified in Indian families with familial hypertrophic cardiomyopathy. J. Mol. Cell. Cardiol. 35, 623–636.

Wally, V., Murauer, E.M., Bauer, J.W., 2012. Spliceosome-mediated trans-splicing: the therapeutic cut and paste. J. Investig. Dermatol. 132, 1959–1966.

Watkins, H., Conner, D., Thierfelder, L., Jarcho, J.A., MacRae, C., McKenna, W.J., Maron, B.J., Seidman, J.G., Seidman, C.E., 1995. Mutations in the cardiac myosin binding protein-C gene on chromosome 11 cause familial hypertrophic cardiomyopathy. Nat. Genet. 11, 434–437.

Weith, A., Sadayappan, S., Gulick, J., Previs, M.J., Vanburen, P., Robbins, J., Warshaw, D.M., 2012. Unique single molecule binding of cardiac myosin binding protein-C to actin and phosphorylation-dependent inhibition of actomyosin motility requires 17 amino acids of the motif domain. J. Mol. Cell. Cardiol. 52, 219–227.

Wessels, M.W., Herkert, J.C., Frohn-Mulder, I.M., Dalinghaus, M., van den Wijngaard, A., de Krijger, R.R., Michels, M., de Coo, I.F., Hoedemaekers, Y.M., Dooijes, D., 2014. Compound Heterozygous or Homozygous Truncating MYBPC3 Mutations Cause Lethal Cardiomyopathy with Features of Noncompaction and Septal Defects. Eur J Hum Genet.

Witayavanitkul, N., Ait Mou, Y., Kuster, D.W., Khairallah, R.J., Sarkey, J., Govindan, S., Chen, X., Ge, Y., Rajan, S., Wieczorek, D.F., Irving, T., Westfall, M.V., de Tombe, P.P., Sadayappan, S., 2014. Myocardial infarction-induced N-terminal fragment of cardiac myosin-binding protein C (cMyBP-C) impairs myofilament function in human myocardium. J. Biol. Chem. 289, 8818–8827.

Witt, C.C., Gerull, B., Davies, M.J., Centner, T., Linke, W.A., Thierfelder, L., 2001. Hypercontractile properties of cardiac muscle fibers in a knock-in mouse model of cardiac myosin-binding protein-C. J. Biol. Chem. 276, 5353–5359.

Woodley, L., Valcarcel, J., 2002. Regulation of alternative pre-mRNA splicing. Brief. Funct. Genomic. Proteomic. 1, 266–277.

Xin, B., Puffenberger, E., Tumbush, J., Bockoven, J.R., Wang, H., 2007. Homozygosity for a novel splice site mutation in the cardiac myosin-binding protein C gene causes severe neonatal hypertrophic cardiomyopathy. Am. J. Med. Genet. A 143A, 2662–2667.

Zacchigna, S., Zentilin, L., Giacca, M., 2014. Adeno-associated virus vectors as therapeutic and investigational tools in the cardiovascular system. Circ. Res. 114, 1827–1846.

Zahka, K., Kalidas, K., Simpson, M.A., Cross, H., Keller, B.B., Galambos, C., Gurtz, K., Patton, M.A., Crosby, A.H., 2008. Homozygous mutation of MYBPC3 associated with severe infantile hypertrophic cardiomyopathy at high frequency among the Amish. Heart 94, 1326–1330.

Zoghbi, M.E., Woodhead, J.L., Moss, R.L., Craig, R., 2008. Three-dimensional structure of vertebrate cardiac muscle myosin filaments. Proc. Natl. Acad. Sci. U. S. A. 105, 2386–2390.