Cardiac Myosin-Binding Protein C Mutations and Hypertrophic Cardiomyopathy: Haploinsufficiency, Deranged Phosphorylation, and Cardiomyocyte Dysfunction
Sabine J. van Dijk, Dennis Dooijes, Cris dos Remedios, Michelle Michels, Jos M.J. Lamers, Saul Winegrad, Saskia Schlossarek, Lucie Carrier, Folkert J. ten Cate, Ger J.M. Stienen and Jolanda van der Velden

Circulation 2009, 119:1473-1483: originally published online March 9, 2009
doi: 10.1161/CIRCULATIONAHA.108.838672
Circulation is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association. All rights reserved. Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at: http://circ.ahajournals.org/content/119/11/1473

Subscriptions: Information about subscribing to Circulation is online at http://circ.ahajournals.org//subscriptions/
Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail: journalpermissions@lww.com
Reprints: Information about reprints can be found online at http://www.lww.com/reprints

Downloaded from http://circ.ahajournals.org/ at Vrije on July 19, 2011
Molecular Cardiology

Cardiac Myosin-Binding Protein C Mutations and Hypertrophic Cardiomyopathy

Haploinsufficiency, Deranged Phosphorylation, and Cardiomyocyte Dysfunction

Sabine J. van Dijk, MSc; Dennis Dooijes, PhD; Cris dos Remedios, PhD; Michelle Michaels, MD, PhD; Jos M.J. Lamers, PhD; Saul Winegrad, PhD; Saskia Schlossarek, PhD; Lucie Carrier, PhD; Folkert J. ten Cate, MD, PhD; Ger J.M. Stienen, PhD; Jolanda van der Velden, PhD

Background—Mutations in the MYBPC3 gene, encoding cardiac myosin-binding protein C (cMyBP-C), are a frequent cause of familial hypertrophic cardiomyopathy. In the present study, we investigated whether protein composition and function of the sarcomere are altered in a homogeneous familial hypertrophic cardiomyopathy patient group with frameshift mutations in MYBPC3 (MYBPC3mut).

Methods and Results—Comparisons were made between cardiac samples from MYBPC3 mutant carriers (n=7; c.2864_2865delCT, n=4) and nonfailing donors (n=13). Western blots with the use of antibodies directed against cMyBP-C did not reveal truncated cMyBP-C in MYBPC3mut. Protein expression of cMyBP-C was significantly reduced in MYBPC3mut by 33±5%. Cardiac MyBP-C phosphorylation in MYBPC3mut samples was similar to the values in donor samples, whereas the phosphorylation status of cardiac troponin I was reduced by 84±5%, indicating divergent phosphorylation of the 2 main contractile target proteins of the β-adrenergic pathway. Force measurements in mechanically isolated Triton-permeabilized cardiomyocytes demonstrated a decrease in maximal force per cross-sectional area of the myocytes in MYBPC3mut (20.2±2.7 kN/m²) compared with donor (34.5±1.1 kN/m²). Moreover, Ca²⁺ sensitivity was higher in MYBPC3mut (pCa₅₀=5.62±0.04) than in donor (pCa₅₀=5.54±0.02), consistent with reduced cardiac troponin I phosphorylation. Treatment with exogenous protein kinase A, to mimic β-adrenergic stimulation, did not correct reduced maximal force but abolished the initial difference in Ca²⁺ sensitivity between MYBPC3mut (pCa₅₀=5.46±0.03) and donor (pCa₅₀=5.48±0.02).

Conclusions—Frameshift MYBPC3 mutations cause haploinsufficiency, deranged phosphorylation of contractile proteins, and reduced maximal force-generating capacity of cardiomyocytes. The enhanced Ca²⁺ sensitivity in MYBPC3mut is due to hypophosphorylation of troponin I secondary to mutation-induced dysfunction. (Circulation. 2009;119:1473-1483.)

Key Words: cardiomyopathy ■ myocardial contraction ■ myocytes ■ mutation ■ proteins

Familial hypertrophic cardiomyopathy (FHC) is the most frequent inheritable cardiac disease with a prevalence of 0.2%.1-2 FHC-causing mutations are identified in 13 genes encoding sarcomeric proteins.3 Mutations in the MYBPC3 gene encoding cardiac myosin-binding protein C (cMyBP-C) represent >40% of all FHC cases.4 Most MYBPC3 mutations are predicted to produce C-terminally truncated proteins, lacking titin and/or major myosin-binding sites.4-6 Studies in FHC patients carrying a MYBPC3 mutation failed to reveal truncated cMyBP-C protein,7-9 suggesting that MYBPC3 mutations may lead to haploinsufficiency.

Clinical Perspective p 1483

Evidence suggests that the first step in the pathogenesis of FHC involves mutation-induced sarcomeric dysfunction.2,3 Myocardial dysfunction in this group of patients has been attributed at least partly to myocyte hypertrophy, disarray, and interstitial fibrosis.10 However, direct evidence for both reduced cMyBP-C expression and sarcomeric dysfunction in MYBPC3 mutant carriers is missing.

Approximately 35% of the FHC patients in the Netherlands have founder mutations in the MYBPC3 gene.
(c.2373dupG and c.2864_2865delCT)\(^{11}\) that both are predicted to encode C-terminally truncated proteins (Figure 1).\(^{8,9}\) This allowed us to investigate whether truncating mutations in the MYBPC3 gene alter sarcomeric protein composition and function in a rather homogeneous patient group. Cardiac MyBP-C mRNA and protein expression and phosphorylation status of sarcomeric proteins were analyzed in concert with cMyBP-C (MYBPC3mut: c.2373dupG, n\(^{11}\)/H\(^{13}\)/H\(^{16}\)6 years; 2/2 male/female), who was moderately depressed (44\(\pm\)2%). Both mutations encode for slightly different C-terminally truncated proteins with a theoretical mass of 93 and 116 kDa, respectively (Figure 1).\(^{15,16}\)

Nonfailing cardiac tissue from the free LV wall was obtained from donor hearts (n\(^{11}\)/H\(^{13}\)/H\(^{16}\)5 years; 10/3 male/female) when no suitable transplant recipient was found. The donor group was slightly younger and included relatively more males than the FHCM group. All samples were immediately frozen and stored in liquid nitrogen. The study protocol was approved by the local ethics committees, and written informed consent was obtained.

### Quantitative mRNA Analyses

Total RNA was extracted from 5 to 40 mg of 4 nonfailing and 4 FHCM frozen cardiac tissues with the use of the SV Total RNA
Protein Analysis
Cardiac samples (11 MYBPC3mut, 8 donor) were treated with trichloroacetic acid before protein analysis to preserve the endogenous phosphorylation status of the sarcomeric proteins.17

Western Immunoblotting
Proteins were separated by 1-dimensional gel electrophoresis on a 15% polyacrylamide SDS gel and subsequently transferred to nitrocellulose paper by wet blotting. Polyclonal antibodies (diluted 1:1000) raised against recombinant C0C2, C5, and C8C9 produced from human cDNA encoding cMyBP-C15 (Figure 1) were used for detection of cMyBP-C (Dr S. Winegrad, University of Pennsylvania, Philadelphia). Primary antibody binding was visualized with a secondary goat anti-rabbit antibody (diluted 1:2000) and enhanced chemiluminescence (Amersham, GE Healthcare, Chalfont St. Giles, UK).

To detect truncated cMyBP-C, 2 antibodies were used, which are directed to the N-terminal part of cMyBP-C (C0C2) and the middle region of cMyBP-C (C5) (Figure 1). The sensitivity of the 2 antibodies to detect low amounts of truncated cMyBP-C under the experimental conditions used was assessed with a dilution series of a nonfailing donor sample (0.04 to 5 μg). The dilution at which the cMyBP-C band was still discernible was defined as the lower detection limit and amounted to 1.6% (0.08 μg) for the C0C2 antibody and 3.2% (0.16 μg) for the C5 antibody (data not shown).

In addition, phosphorylation of cMyBP-C at Ser23/24 (ie, protein kinase A [PKA] sites, rabbit polyclonal antibody; detection limit and amounted to 1.6% (0.08 μg) for the C0C2 antibody and 3.2% (0.16 μg) for the C5 antibody (data not shown). The WT probe recognized a WT sequence in exon 27 (F5′-TGG AGC CCC TGT TAC CAC C-3′), and the mutant probe recognized a mutant sequence (deleted of CT) in exon 27 (F5′-TGCG GCC GGG ACA CAA TAT G-3′) and 25 (R5′-AGC TCC ACC GGT AGC TCT TCT TC-3′). Specific TaqMan probes were designed to recognize either the WT mRNA in exon 24 (F5′-GAG CCG CCT GCC TAC GAT-3′) or the mutant mRNA at the junction between the smaller exon 24 (−40 hp due to the new cryptic donor splice site) and exon 25 (F5′-GCA CAG TAC AGG CTA CAT CCT G-3′). For the c.2864_2865delCT mutation, primers were designed in exons 27 (F5′-AGT GCG GGC ACA CAA C-3′) and 28 (R5′-GGG ATG AGA AGG TTC ACA GG-3′). The WT probe recognized a WT sequence in exon 24 (F5′-TGG AGC CCC TGT TAC CAC C-3′), and the mutant probe recognized a mutant sequence (deleted of CT) in exon 27 (F5′-CTG GAG CCC GTT ACC ACC A-3′). GAPDH was used as endogenous control to normalize the quantification of the target mRNAs for difference in the amount of cDNA added to each reaction. All analyses were performed in triplicate with the software ABI 7900HT SDS 2.2. The mRNA amount was estimated according to the comparative Ct method with the 2-ΔΔCt formula. The amount of both WT and mutant mRNA was reported as the mean of the WT obtained from the 4 nonfailing samples for each exon amplification.

Isomeric Force Measurements
Cardiomyocytes were mechanically isolated from small tissue samples as described previously.18 Triton-permeabilized cardiomyocytes were glued between a force transducer and a piezoelectric motor and stretched to a sarcomere length of ~2.2 μm. Force measurements were performed at various calcium concentrations (pCa, −log[Ca2+], values ranging from 4.5 to 6.0) as described previously.18,19 Force measurements were performed in single cardiomyo-
cytes isolated from 10 MYBPC3mut (34 cardiomyocytes) and 13 donor samples (47 cardiomyocytes). On average, 2 to 5 cardiomyocytes were studied for each patient/donor. One MYBPC3mut biopsy was too small to isolate cardiomyocytes and was used for protein analysis only. Cross-sectional area (width/depth/4) of the cardiomyocytes determined at a sarcomere length of 2.2 \( \mu \text{m} \) was significantly higher in MYBPC3mut (508\( \pm \)67 \( \mu \text{m}^2 \)) than in donor (374\( \pm \)26 \( \mu \text{m}^2 \)). Length between the attachments did not significantly differ and amounted to 62\( \pm \)5 \( \mu \text{m} \) in MYBPC3mut and 72\( \pm \)4 \( \mu \text{m} \) in donor. Passive tension (F_{passive}) was determined by shortening the cell in relaxation solution (pCa 9.0) by 30% and immediately restretching it to its original length. Maximal calcium activated tension (F_{active} [ie, maximal force/cross-sectional area]) was calculated by subtracting F_{passive} from the total force (F_{total}) at saturating [Ca\(^{2+}\)] (pCa 4.5). Ca\(^{2+}\) sensitivity is denoted as pCa\(_{50}\) (ie, pCa value at which 50% of F_{active} is reached). Force measurements were repeated after incubation of cells for 40 minutes at 20°C in relaxing solution containing the catalytic subunit of PKA (100 U/mL, Sigma) or with the catalytic domain of protein kinase C (PKC) (0.25 U/mL, Sigma).

Data Analysis
Data are presented as mean\( \pm \)SEM. Cardiomyocyte force values were averaged per sample, and mean values for MYBPC3mut and donor samples were compared with unpaired Student t tests. Effects of PKA/PKC were tested with 2-way ANOVA. \( P<0.05 \) was considered significant. Asterisks denote significant difference between MYBPC3mut and donor, and daggers denote significant difference before versus after PKA/PKC treatment.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Reduced Mutant cMyBP-C mRNA Level in FHCM Ventricular Tissue
To investigate whether both WT and mutant cMyBP-C mRNAs were transcribed in FHCM ventricular tissues, real-time reverse transcription polymerase chain reaction was performed with the use of specific Taqman probes and primers for each mutation (Figure 2A). The c.2373dupG mutation was expected to induce a cryptic donor splice site in exon 24 and the skipping of 40 nucleotides (Figure 1B). We therefore designed WT and mutant probes accordingly. Similarly, a specific mutant probe was designed for the 2864 to 2865 CT deletion in exon 27 and the corresponding WT probe (Figure 2A). Mutant mRNA represented 23% and 20% of the total cMyBP-C mRNA in c.2373dupG and c.2864_2865delCT groups, respectively (Figure 2B). Because both mutations result in a frameshift and a premature termination codon (Figure 1B), the data suggest that both nonsense mutant mRNAs are partially subjected to degradation by the nonsense-mediated mRNA decay.20

Reduced cMyBP-C Protein Level in FHCM Ventricular Tissue
The presence of truncated cMyBP-C in MYBPC3mut patients was examined by Western immunoblotting. After separation by gel electrophoresis, proteins were transferred to nitrocellulose and visualized with Ponceau (Figure 3A). Figure 3B
shows results with the use of an antibody directed against the C0C2 region of cMyBP-C. None of the antibodies used (against C0C2, C5, or C8C9) revealed truncated cMyBP-C (predicted mass at 93 or 116 kDa) in any of the samples. On the basis of the sensitivity of our Western immunoblot analysis, levels of truncated cMyBP-C/α11021 could not be detected. Hence, we cannot completely exclude the presence of trace amounts of truncated cMyBP-C in MYBPC3mut. However, overloading of MYBPC3mut samples (40 μg; 8 × higher concentration) did not reveal protein bands at the predicted mass (not shown), indicating the trace amounts of truncated protein, if present, would be even <0.2%.

To determine the levels of full-length cMyBP-C, proteins were separated by 1-dimensional gel electrophoresis and stained with SYPRO Ruby (Figure 3C). The α-actinin intensity was used as loading control. The cMyBP-C/α-actinin protein ratio on the SYPRO Ruby–stained gels was 33% lower in MYBPC3mut (n=11) than in donors (n=8) (Figure 3D). Western immunoblot analysis confirmed a reduced level of full-length cMyBP-C (Figure 3B) in MYBPC3mut compared with donor myocardium. On average, Western blot data with the use of the C0C2 antibody showed a 23% lower amount of cMyBP-C (normalized to Ponceau-stained actin on the same blot) in MYBPC3mut compared with donor. However, the coefficient of variation of the Western immunoblot analysis was much higher than that of SYPRO Ruby staining (56.3% and 25.8%, respectively). Therefore, the SYPRO values are considered to represent the protein levels more accurately.

**Deranged Phosphorylation**

The SYPRO Ruby–stained gels were also stained with ProQ Diamond, which selectively stains phosphorylated serine, threonine, and tyrosine residues (Figure 4A). Phosphorylation of cMyBP-C normalized to α-actinin was reduced by 47±7% in the MYBPC3mut compared with donor myocardium (P<0.0001; Figure 4B). Interestingly, phosphorylation of cMyBP-C normalized to its own protein level (determined with SYPRO) was similar between MYBPC3mut and donor myocardium (79±11% versus 100±5%, respectively; P=0.14; Figure 4C).

In addition, massive dephosphorylation of cTnI was observed in MYBPC3mut relative to donor by 84±5% (P<0.0001; Figure 4D). Because the reduced ProQ Diamond signals for cTnI in MYBPC3mut may be due to reduced cTnI expression, we analyzed the steady state level of cTnI by Western immunoblot. The amount of cTnI relative to Ponceau-stained actin did not differ between MYBPC3mut and donor myocardium. *P<0.05.

---

**Figure 4.** Phosphorylation status of cMyBP-C and cTnI. A, ProQ Diamond–stained gradient gel. MWM indicates molecular weight marker. B, The phosphorylation status of cMyBP-C (relative to SYPRO Ruby–stained α-actinin) is lower in MYBPC3mut (n=11) than in donors (n=8), whereas phosphorylation of cMyBP-C normalized to its own expression level is comparable between MYBPC3mut and donors (C). D, The phosphorylation status of cTnI (relative to SYPRO Ruby–stained α-actinin) is lower in MYBPC3mut than in donors. E, cTnI relative to Ponceau-stained actin did not differ between MYBPC3mut and donor myocardium. *P<0.05.
donor myocardium (Figure 4E). Hence, the reduced ProQ Diamond signals represent reduced phosphorylation of cTnI in MYBPC3mut compared with donor. Phosphorylation of other sarcomeric proteins desmin, cardiac troponin T, and myosin light chain 2 was also significantly reduced in MYBPC3mut by 24±8%, 41±7%, and 61±4%, respectively, relative to donor samples.

Western immunoblot analysis (Figure 5) revealed significantly lower bisphosphorylation of PKA sites (Ser23/24) in cTnI in MYBPC3mut compared with donors, whereas phosphorylation of cMyBP-C at Ser282 was similar in MYBPC3mut and donor myocardium. This confirms the data obtained with ProQ Diamond stain (Figure 4).

Depressed Force Development

Functional implications of the MYBPC3 mutations were investigated by cardiomyocyte force measurements in c.2373dupG (n=7; 25 cells) and c.2864_2865delCT (n=3; 9 cells). A representative cardiomyocyte and a recording at saturating Ca2+ concentration (pCa 4.5) from a MYBPC3mut patient are shown in Figure 6A and 6B. Maximal force development (Factive) was significantly depressed in MYBPC3mut (20.2±2.7 kN/m²) compared with donor (34.5±1.1 kN/m²; n=13; 47 cells) (Figure 6C). Passive force (Fpassive) in MYBPC3mut (3.8±0.7 kN/m²) was somewhat elevated compared with donor (3.4±0.4 kN/m²), but the difference was not significant (Figure 6D). Interpatient variation in Factive and Fpassive in the MYBPC3mut group was larger than in the donor group (Figure 6E, 6F). Factive and Fpassive were lower in c.2864_2865delCT than in c.2373dupG (P=NS).

Cells from MYBPC3mut (c.2373dupG, n=4, 9 cells; c.2864_2865delCT; n=2; 6 cells) and donors (n=6; 18 cells) were incubated with PKA, after which Factive and Fpassive measurements were repeated to determine whether β-adrenergic stimulation could correct depressed force in MYBPC3mut. PKA treatment resulted in a minor decrease in Factive in MYBPC3mut (before versus after: 14.2±2.9 and 13.5±2.5 kN/m²), which was similar to the PKA effect in donor (before versus after: 27.9±3.5 and 25.8±3.2 kN/m²) (P=0.03, 2-way ANOVA). Moreover, PKA significantly decreased Fpassive in both groups (MYBPC3mut before versus after: 2.3±0.3 and 2.1±0.2 kN/m²; donor before versus after: 3.3±0.5 and 2.7±0.4 kN/m²) (P=0.001, 2-way ANOVA).

Because increased PKC activity/expression has been associated with depressed maximal force generating capacity of myofilaments, force measurements were repeated after incubation with the catalytic domain of PKC. Factive in MYBPC3mut (c.2373dupG; n=3; 5 cells) was significantly reduced after PKC by 2.6±0.6 kN/m². A similar decrease (3.3±0.7 kN/m²) was found in cardiomyocytes from donor hearts (n=5; 9 cells). PKC slightly decreased Fpassive in both groups, although the effect was not significant (data not shown).

Enhanced Ca2+ Sensitivity

Ca2+ sensitivity of the sarcomeres was significantly higher in MYBPC3mut (pCa0=5.62±0.04) than in donor cells (pCa0=5.54±0.02). The average force-pCa relationships obtained in MYBPC3mut and donor cardiomyocytes are shown in Figure 7A. Ca2+ sensitivity was similar in c.2864_2865delCT (pCa0=5.63±0.05) and c.2373dupG (pCa0=5.60±0.09). Figure 7B illustrates that interpatient variation in pCa0 was larger in the MYBPC3mut than in the donor group.

Treatment with exogenous PKA significantly reduced Ca2+ sensitivity in both groups. The reduction in pCa0 was significantly larger in MYBPC3mut (ΔpCa0=0.18±0.03) than in donor (ΔpCa0=0.06±0.01) cells (Figure 7C). PKA treatment abolished the initial difference in Ca2+ sensitivity between MYBPC3mut and donor (Figure 7D). ProQ Diamond staining of a MYBPC3mut sample that was incubated without...
(control incubation) and with PKA showed a 4-fold increase in cTnI phosphorylation, whereas the increase in cMyBP-C phosphorylation was small (Figure 7E).

Similar to PKA, PKC significantly reduced pCa50 in both groups. However, in contrast to PKA, the PKC-induced shift in pCa50 did not significantly differ between MYBPC3mut (ΔpCa50 = 0.11 ± 0.04) and donor cells (ΔpCa50 = 0.08 ± 0.01) and thus does not explain the baseline difference in Ca2+ sensitivity between MYBPC3mut and donor myocardium.

**Discussion**

Our study provides direct evidence for reduced cMyBP-C protein level and contractile dysfunction in a group of FHCM patients with MYBPC3 frameshift mutations. Consistent with previous studies,7–9 no truncated cMyBP-C protein was detected, and the amount of full-length cMyBP-C was 33% lower in FHCM than in donor myocardium. Our data therefore indicate that the pathomechanism involves haploinsufficiency rather than a poison polypeptide. Using adenoviral gene transfer of cardiomyocytes, Sarikas et al23 showed rapid and quantitative degradation of truncated forms of cMyBP-C by the ubiquitin-proteasome system, which could in turn inhibit ubiquitin-proteasome system–mediated degradation of other cellular proteins.23 Thus, the absence of truncated cMyBP-C in FHCM patients in the present study suggests that the ubiquitin-proteasome system may degrade truncated cMyBP-C. The full-length C-protein, in contrast, compensates for the absence of truncated protein. The fact that mutant mRNAs were detected in both FHCM groups supports the involvement of the ubiquitin-proteasome system in the degradation of truncated protein. On the other hand, the lower level of mutant versus WT cMyBP-C mRNA in both FHCM groups suggests partial instability of nonsense mutant mRNA, which could be degraded by the nonsense-mediated mRNA decay.20

The maximum force-generating capacity (ie, Factive) of cardiomyocytes from MYBPC3mut carriers was significantly reduced by 42% compared with nonfailing myocardium. Recent studies24–28 revealed an important role for cMyBP-C in cross-bridge kinetics. Loss of cMyBP-C accelerates cross-bridge cycling and impairs kinetics of contraction and relaxation.24,25,27 Complete knockout of cMyBP-C resulted in profound cardiac hypertrophy and impaired contractile function in mice.29,30 Surprisingly, transgenic mice harboring only 40% of the normally expressed full-length cMyBP-C did not have LV hypertrophy and showed preserved cardiac function.31 In contrast, our study shows that an ~33% reduction of full-length cMyBP-C level is sufficient to trigger LV hypertrophy and contractile dysfunction in human. Intriguingly, reduced cMyBP-C levels per se do not seem to explain the decline in maximum force in MYBPC3mut because Factive
did not correlate with the level of full-length cMyBP-C (not shown) but may rather involve reduced expression and altered phosphorylation of cMyBP-C.

Cardiac MyBP-C is phosphorylated by PKA on adrenergic stimulation. Apart from PKA, cMyBP-C can be phosphorylated by Ca\(^{2+}\)-calmodulin-dependent kinase (CaMK) and PKC. Transgenic mice hearts in which the phosphorylation sites of cMyBP-C were changed to nonphosphorylatable alanines displayed reduced contractility and altered sarcomeric structure, indicating that phosphorylation of cMyBP-C is essential for normal cardiac function. Reduced cMyBP-C phosphorylation has been observed in animal models of cardiac hypertrophy and failure and in humans with end-stage idiopathic and ischemic cardiomyopathy. The discrepant phosphorylation levels of cMyBP-C and cTnI in MYBPC\(_{3\text{mut}}\) are in contrast to previous observations in non-FHCM (idiopathic and ischemic cardiomyopathy) and donor myocardium, which revealed parallel changes in the main target proteins of the \(\beta\)-adrenergic pathway. Hence, it is possible that this discrepancy causes contractile dysfunction. Because PKA did not correct the reduction in \(F_{\text{active}}\), other (mal)adaptive signaling routes are responsible for divergent phosphorylation of cMyBP-C and cTnI and sarcomeric dysfunction. In a recent study, increased PKC expression level in 2 models of heart failure (pressure overload and ischemic) in rat was associated with reduced maximal force-generating capacity of myofilaments. To test whether this applies to human tissue, force measurements were performed in single human cardiomyocytes from MYBPC\(_{3\text{mut}}\) and donor hearts before and after incubation with PKC. The effects of PKC on cardiomyocyte force parameters (\(F_{\text{active}}, F_{\text{passive}},\) and \(pC_{50}\)) were similar in MYBPC\(_{3\text{mut}}\) and donor. ProQ Diamond staining of MYBPC\(_{3\text{mut}}\) myocardium, which was directly frozen or incubated without PKA (CTRL indicates control) or with PKA (100 U/mL). Phosphorylation of cTnI increased on incubation with PKA. cTnT indicates cardiac troponin T; MLC-2, myosin light chain 2.

Figure 7. A, Force measurements were performed at maximal and submaximal [Ca\(^{2+}\)] (pCa range 4.5 to 6). Force values obtained in solutions with submaximal [Ca\(^{2+}\)] were normalized to the maximal force level at pCa 4.5. Force-pCa relations were fit to a modified Hill equation. Ca\(^{2+}\) sensitivity of the sarcomeres (pCa\(_{50}\)) was significantly higher in MYBPC\(_{3\text{mut}}\) than in donor. B, Variation among mean pCa\(_{50}\) parameters of individuals among the MYBPC\(_{3\text{mut}}\) group (c.2373dupG, black symbols; c.2864_2865delCT, gray symbols) compared with the donor group (open symbols). C, Treatment of cardiomyocytes with exogenous PKA induced a larger reduction in pCa\(_{50}\) in MYBPC\(_{3\text{mut}}\) than in donor. D, PKA treatment abolished the initial difference in Ca\(^{2+}\) sensitivity between MYBPC\(_{3\text{mut}}\) and donor. *P < 0.05 MYBPC\(_{3\text{mut}}\) vs donor; †P < 0.05 before vs after PKA treatment. E, ProQ Diamond staining of MYBPC\(_{3\text{mut}}\) myocardium, which was directly frozen (BL indicates baseline) or incubated without PKA (CTRL indicates control) or with PKA (100 U/mL). Phosphorylation of cTnI increased on incubation with PKA. cTnT indicates cardiac troponin T; MLC-2, myosin light chain 2.
maximal force-generating capacity of cardiomyocytes is not a direct consequence of haploinsufficiency but rather might be caused by differential phosphorylation of cMyBP-C resulting from (mal)adaptive neurohumoral signaling in hearts of MYBPC3

Similarly, the higher Ca\(^{2+}\) sensitivity of force development in MYBPC3

This study was supported by ICaR-VU PhD fellowship (2007 to Dr van der Velden), the sixth Framework Program of the European Union (Marie Curie EXT-014051 to Dr Carrier), and the Deutsche Forschungsgemeinschaft (FOR-604/1, CA 618/1-2 to Dr Carrier).

Saskia Schlossarek is a postdoctoral fellow from the DFG-FOR-604/1 grant.

Disclosures

References


Sources of Funding

Acknowledgments

We thank D.H. Dekkers (Erasmus Medical Center, Rotterdam, the Netherlands) for his assistance with sample collection.

None.

This study was supported by ICaR-VU PhD fellowship (2007 to Dr van der Velden), the sixth Framework Program of the European Union (Marie Curie EXT-014051 to Dr Carrier), and the Deutsche Forschungsgemeinschaft (FOR-604/1, CA 618/1-2 to Dr Carrier).


42. Hofmann PA, Hartzell HC. Miss RL. Alterations in Ca2+-sensitive tension due to partial extraction of C-protein from rat skinned cardiac myocytes and rabbit skeletal muscle fibers. J Gen Physiol. 1991;97:1141–1163.


Familial hypertrophic cardiomyopathy is the most frequent inheritable cardiac disease, with a prevalence of 1/500. Mutations in the MYBPC3 gene encoding cardiac myosin-binding protein C (cMyBP-C) represent >40% of all familial hypertrophic cardiomyopathy cases. Cardiac MyBP-C is bound to the thick myosin filament and may influence binding of the myosin head to actin and thereby force generation and pressure development of the heart. cMyBP-C function is influenced by β-adrenergic stimulation through protein kinase A–mediated phosphorylation. Most MYBPC3 mutations are predicted to produce C-terminally truncated proteins, lacking titin and/or major myosin binding sites. Approximately 35% of the familial hypertrophic cardiomyopathy patients in the Netherlands have founder mutations in the MYBPC3 gene that encode C-terminally truncated proteins. This allowed us to investigate whether truncating mutations in MYBPC3 alter sarcomeric protein composition and function in a rather homogeneous patient group. No truncated cMyBP-C was detected, full-length cMyBP-C was reduced by 33% compared with nonfailing myocardium, and cMyBP-C phosphorylation was preserved. Our data therefore indicate that the pathomechanism involves haploinsufficiency rather than a poison polypeptide. Force measurements in single cardiomyocytes revealed reduced maximal force-generating capacity compared with healthy cells. In addition, Ca2⁺ sensitivity of the contractile apparatus was increased because of hypophosphorylation of troponin I, another target of protein kinase A. We conclude that the sarcomeric phenotype in familial hypertrophic cardiomyopathy with MYBPC3 mutations includes a primary contractile sarcomeric defect causing deranged secondary alterations in protein phosphorylation. Our data therefore suggest that contractile dysfunction is a pivotal link between the mutant sarcomeric protein and pathological hypertrophic cardiomyopathy.