J. of Cardiovasc. Trans. Res. (2009) 2:441–451
DOI 10.1007/s12265-009-9131-8

# Mechanical and Energetic Consequences of HCM-Causing Mutations

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Received: 17 July 2009 / Accepted: 15 September 2009 / Published online: 9 October 2009


**Abstract** Hypertrophic cardiomyopathy (HCM) was the first inherited heart disease to be characterized at the molecular genetic level with the demonstration that it is caused by mutations in genes that encode different components of the cardiac sarcomere. Early functional in vitro studies have concluded that HCM mutations cause a loss of sarcomere mechanical function. Hypertrophy would then follow as a compensatory mechanism to raise the work and power output of the affected heart. More recent in vitro and mouse model studies have suggested that HCM mutations enhance contractile function and myofilament $Ca^{2+}$ sensitivity and impair cardiac myocyte energetics. It has been hypothesized that these changes may result in cardiac myocyte energy depletion due to inefficient ATP utilization and also in altered myoplasmic $Ca^{2+}$ handling. The problems encountered in reaching a definitive answer on the effects of HCM mutations are discussed. Though direct analysis of the altered functional characteristics of HCM human cardiac sarcomeres has so far lagged behind the in vitro and mouse studies, recent work with mechanically isolated skinned myocytes and myofibrils from affected human hearts seem to support the energy depletion hypothesis. If further validated in the human heart, this hypothesis would identify tractable therapeutic targets that suggest that HCM, perhaps more than any other cardiomyopathy, will be amenable to disease-modifying therapy.

**Keywords** Hypertrophic Cardiomyopathy · Myosin · Myosin Binding Protein-C · Cardiac Muscle

Familial hypertrophic cardiomyopathy (HCM) is an autosomal dominant inherited heart disease, which results in hypertrophy of the left ventricle in the absence of other cardiac or systemic disease (such as hypertension or aortic stenosis). It is characterized morphologically by left ventricular wall and septal hypertrophy, resulting in a decreased left ventricle volume. Obstruction of the left ventricular outflow tract is present at rest in approximately 25% of patients and in a higher proportion during exercise. Histologically, areas of myocyte disarray and interstitial fibrosis are characteristic of the disease. Although systolic contractility is most often preserved and features of hypercontractility are observed at the whole heart level, outflow obstruction and impaired relaxation can cause progressive heart failure and increased incidence of ventricular arrhythmias can lead to sudden cardiac death.

## Molecular Basis of HCM: A Disease Intrinsic to the Cardiac Sarcomere

In 1989, HCM was mapped to chromosome 14 in a French–Canadian pedigree with autosomal dominant disease [24]. In 1990, the first missense mutation in a sarcomeric protein, R403Q in the β-cardiac myosin heavy chain (β-MyHC), was shown to be responsible for HCM [17]. Since then, molecular genetics has revealed that HCM is a complex molecular disease exhibiting both gene and

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allele heterogeneity (multiple disease genes and multiple mutations) with as many as ten sarcomere-related genes and more than 400 predominantly missense mutations described. Besides β-MyHC, the disease genes (Fig. 1) encode (1) other thick filament proteins: cardiac myosin-binding protein-C (cMyBP-C also named cardiac C-protein) [8, 70], both regulatory (RLC), and essential (ELC) myosin light chains [48], (2) thin filament proteins: α-tropomyosin (α-Tm) [65], cardiac troponin T (cTnT) [65], cardiac troponin I (cTnI) [28], cardiac troponin C (cTnC) [21, 31], actin [39], and (3) the giant protein titin [56] which spans both the thick and thin filaments. Most mutations appear to act as dominant negatives and the mutant proteins are incorporated into the sarcomere where they can affect contractile performance (e.g., [12, 10]). A significant exception to this is likely to be the numerous truncation mutations in cMyBP-C for which evidence of haplo-insufficiency has been recently generated [68, 69].

Disease-causing mutations in sarcomere proteins are detected in 60–70% of HCM-affected individuals and the most commonly affected genes are MYBPC3 (cMyBP-C) and MYH7 (β-MyHC), together accounting for approximately 80% of identified mutations [50].

The prevalence of mutations in sarcomeric proteins has led to the designation of HCM as primarily a "disease of the sarcomere" [65, 57, 37]. This raises the question of how molecular defects in sarcomeric proteins can lead to myofibril disarray, asymmetric left ventricular hypertrophy, diastolic dysfunction, and an increased probability of sudden death. The question is especially challenging also because the clinical phenotypes among patients with the same mutation can be rather heterogeneous.

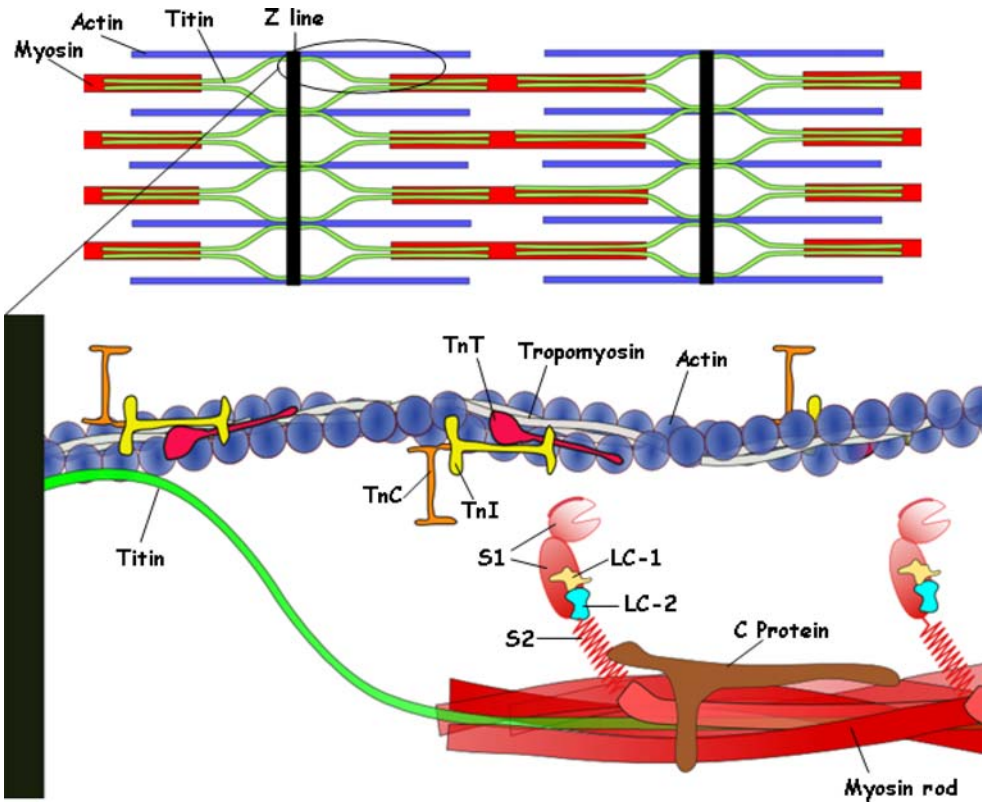


**Fig. 1** The cardiac sarcomere. The cardiac sarcomere comprises the proteins of the thick (*red*) and thin (*blue*) filaments, and the giant protein titin (*green*) connecting thick filaments to the *Z lines*. Mutations in genes encoding each of the proteins labeled in the figure can cause hypertrophic cardiomyopathy (HCM). The disorder can thus result from alterations of proteins that have diverse motor, regulatory, and structural functions. Myosin heavy chain (MyHC), the motor of cardiac contraction (it is a dimer but in the scheme is depicted as a single-headed monomer), is made of different domains: the *myosin rod* composing the backbone of thick filaments, *S2* (arbitrarily depicted as an elastic spring), connecting the rod to the myosin head, and the myosin head *S1* comprising the actin-binding, catalytic domain, and the light chain-binding domain (the lever arm of the motor protein). Most HCM mutations in MyHC are in *S1*, but HCM-associated mutations have been also described in both *S2* and the *myosin rod*. The other thick filament-associated proteins are the essential (ELC or *LC-1*) and the regulatory (RLC or *LC-2*) myosin light chains bound to the lever arm of *S1*, and *C-protein* (or cardiac myosin binding protein-C, cMyBP-C), a protein with poorly understood regulatory and structural functions. Thin filament proteins comprise, besides actin, the troponin complex (troponin C, TnC, troponin I, TnI, and troponin T, TnT) and tropomyosin

## Disease Mechanisms in Sarcomeric HCM: Loss or Gain of Function?

Since the discovery of the first HCM-associated mutation in β-MyHC, a large number of biochemical and biophysical studies have been published on the effects of HCM mutations on the functional properties of the mutant proteins. Rather than summarize these many studies for each gene and mutation, we will focus mostly on results obtained for the single most extensively studied myosin mutation, namely, R403Q.

The R403Q mutation replaces a basic arginine residue with a polar glutamine residue at the base of a highly conserved actin-binding loop, renamed the cardiomyopathy loop because of its role in HCM. The effects of this amino-acid replacement on the properties and functions of myosin are not predictable either in terms of actin binding or any change in the energetic cost of contraction.

Early studies of systems containing the R403Q mutant myosin [12, 61, 32, 55, 15, 53] showed severely diminished function, whatever sources of mutant protein were used (biopsies from slow skeletal muscle of HCM patients or recombinant proteins from different expression systems) and whatever assays were performed (in vitro motility assays and acto-myosin ATPase in isolated proteins, force, and shortening velocity in slow skeletal skinned muscle fibers). On the basis of these results, it has been hypothesized that HCM is caused by altered acto-myosin interactions and that the accompanying loss of sarcomere function drives the hypertrophy of the left ventricle [12, 36].

Although intuitively appealing, the "compensatory hypertrophy hypothesis" for HCM, has been challenged by a number of studies on the effects of HCM mutations in sarcomeric proteins other than β-MyHC and more recent work on the R403Q mutation. Most (though not all) HCM mutations in thin filament regulatory proteins (α-Tm, cTnI, cTnT, cTnC) increase the $Ca^{2+}$ sensitivity of force generation, thereby increasing force at submaximal $Ca^{2+}$ concentrations that are normal in intact cardiac myocytes (e.g. [10, 41, 14, 45]). In addition, actin filaments, when reconstituted with regulatory proteins containing some HCM-associated TnT mutants, show increased velocity of translocation in in vitro motility assays [33, 60, 23]. Finally, studies of novel systems containing the R403Q and other HCM-mutant myosins imply that the mutant proteins have enhanced motor activity rather than diminished function (e.g., [72, 66, 38]. For example, using homozygous R403Q myosin isolated from neonatal transgenic mice (in the α-MyHC backbone) [66] found higher sliding velocity in an in vitro motility assay along with higher actin-activated ATPase activity. Evidence of an apparent gain in motor protein function has been also obtained from cardiac samples of R403 mutant HCM patients [27, 7]; see also below).

To reconcile the lack of consistent contractile depression in HCM, it has been proposed that HCM sarcomere mutations may lead to increased cost of force production through inefficient or excessive ATP usage and that this ultimately results in an energy deficiency that contributes to the pathogenesis of the disease [2, 3]. The "energy depletion hypothesis" for HCM is strengthened by the finding that HCM-like phenotypes have been reported in a variety of diseases that limit myocardial energy production. Interestingly, mutations in the γ2 regulatory subunit of AMP-activated protein kinase, which senses and protects cells from ATP depletion, cause cardiomyopathy with severe hypertrophy (more than can be accounted for by the glycogen deposition), and ventricular pre-excitation [6, 1]. Furthermore, it has been shown, using nuclear magnetic resonance ($^{31}$P NMR) spectroscopy, that the cardiac phosphocreatine (PCr) to ATP ratio, a measure of energy status, is reduced in HCM patient hearts by ~30% compared with controls [11]. The PCr/ATP ratio was reduced in both those with and without left ventricular hypertrophy suggesting that energy deficiency is a primary event rather than a secondary consequence of remodeling.

One of the predicted consequences of the energy depletion hypothesis for HCM is that reuptake of $Ca^{2+}$ into the sarcoplasmic reticulum will be compromised because of the extreme energy requirements of the cardiac SERCA pump (Sarco-Endoplasmic Reticulum Calcium ATPase). In support of this notion, $^{31}$P NMR spectroscopy studies in transgenic mouse models of HCM have shown that the ΔG of ATP hydrolysis is reduced to a level at which SERCA function will be compromised [58, 26, 19]. Increased $Ca^{2+}$ concentrations in specific microdomains would activate hypertrophy-signaling pathways.

In addition, these proposed mechanisms, predicted to yield increased cytosolic $Ca^{2+}$, would be pro-arrhythmic. Also, the increased myofilament $Ca^{2+}$ sensitivity, frequently reported in HCM models, can be related to increased susceptibility to cardiac arrhythmias [4]. As myofilaments are the major dynamic buffer of $Ca^{2+}$ in the sarcoplasm, it is likely that this will impact on the $Ca^{2+}$ transient. This, in turn, may lead to action potential remodeling and further alterations in intracellular $Ca^{2+}$ handling to create both an arrhythmogenic substrate and a trigger. In isolated mouse hearts, myofilament $Ca^{2+}$-sensitization by drugs or troponin mutations associated with HCM are reported to induce arrhythmias by (1) shortening the effective refractory period and the action potential duration, (2) slowing conduction velocity, and (3) predisposing to early after-depolarizations and triggered activity [4].

## Disparate Models, Disparate Results

Although increased energy cost of tension generation may be central to the HCM disease process and helps explain

some unresolved clinical observations [2], the pathways linking the various genetic defects to the characteristic human disease phenotype remain largely unknown. Even the fundamental question of whether these mutations cause a gain or loss of sarcomere mechanical function remains unresolved.

The effects of the R403Q mutation on myosin function show that substantial disagreement exists between early and subsequent reports on this topic (literature cited above). A novel report [35] suggests that the functional sequelae of the R403Q mutation are dependent on the context of the cardiac myosin isoform, adding further complexity. A limitation of the original R403Q murine model for HCM [16] is that the mutation was produced by replacing, by homologous recombination, the gene of the mouse α-cardiac MyHC, the predominant myosin isoform in the postnatal mouse ventricle with the mutant α-cardiac MyHC *R403Q* gene. All larger mammals, including humans, have the slower β-cardiac myosin expressed predominantly in their ventricles, and the faster α-MyHC is confined to the atria. Because the α- and β-cardiac MyHC isoforms of all species are 93% identical in amino-acid sequence, it has been generally assumed that the different backbones would have few, if any, consequences for function. This assumption has been tested using a transgenic mouse model in which the endogenous α-cardiac MyHC was largely replaced with transgenically encoded β-cardiac MyHC and comparing the functional properties of R403Q myosin expressed in an α- or β-backbone [35]. Whereas the mutation in an α-backbone showed an increase in sliding velocity in the in vitro motility assay and actin-activated ATPase activity relative to wild-type α-myosin, confirming previous findings ([66], see above), the same mutation in a β-backbone had little effect on motility and a slight decrease of enzymatic activity relative to wild-type β-myosin.

Why are there such disparate results on the functional sequelae of sarcomeric protein mutations associated with HCM? Answers may lie in the different assessment methods for protein function and in different sources of mutant proteins.

Function of sarcomeric protein has been assessed (1) in vitro in isolated proteins or (2) in situ in myocytes (and other isolated muscle models) or in intact hearts expressing the mutant proteins. Sarcomeric protein dysfunction can be studied in situ by measuring the mechanical parameters of contractility in myocytes or diastolic and systolic function of the intact heart, most often, from transgenic mouse models. One major problem associated with in situ studies is that secondary changes in myocardial structure and other myocyte proteins besides the mutant one can complicate the interpretation of results. Many in vitro studies have used the thin filament sliding motility assay that tracks the movement of fluorescently labeled actin filaments (unregulated or reconstituted with regulatory proteins) generated by myosin molecules adsorbed onto a fixed surface. Actin-activated ATPase activity can also be measured in isolated proteins. One should consider that these assays probe the acto-myosin transduction cycle and its regulation under unloaded conditions and in the absence of the structured sarcomere environment. In addition, all these assays relay on the quality of the protein preparations and this, especially for myosin, may be problematic.

In fact, the source and quality of material for in vitro and in situ studies has likely been a major reason for the variability between studies of the mutant proteins. Limited purification methods of small amounts of myosin from human biopsies may have resulted in inadequate experimental material in the earliest studies on the R403Q mutation [44, 34]. The source of material in subsequent studies has largely been either recombinant proteins (either studied in vitro or exchanged—or expressed by transfection—into a variety of muscle preparations) or engineered mouse models.

It now appears that using recombinant techniques to introduce cardiac myosin mutations into truncated or smooth muscle myosins or into fast-type mouse and rat myosins, even if technically perfect, may not faithfully mimic the human mutant myosin [34, 35]. While these molecules may be useful for investigating the physiology of the sarcomere, they may produce misleading results in the context of identifying the disease mechanisms in HCM. Work with recombinant proteins other than myosin has also shown that the effect of a mutation is often very context-dependent, being influenced by isoform type, species of origin, and posttranslational modifications especially phosphorylation [5, 13]. Investigations of the effects of admixture of mutant and wild-type proteins have shown the importance of studying 50:50 mixtures, as are expected in a heterozygous disease [49, 52, 51].

A number of over-expressing transgenic mouse models and some gene-targeted lines have been created and characterized (for a review see Shephard and Semsarian "Role of animal model in HCM research" in this issue). These models have had great impact on our knowledge of disease–gene relationships and HCM pathogenesis. Moreover, the use of novel inducible transgenic mouse models of HCM (the transgene of interest can be turned on at preselected times) will greatly facilitate our understanding of the primary functional effects of HCM mutations and the factors which may induce and modify the progression of the disease. Not all of these transgenic models, however, have mimicked the human HCM phenotype and in some instances, this may reflect a stoichiometry of mutant/wild-type protein that is not representative of human HCM. In many transgenic HCM models, the biophysical changes at the level of the myofilament closely mirror those seen in other experimental systems, but the whole organ phenotype

does not mirror human disease (e.g., no hypertrophy or increased risk of sudden death), perhaps reflecting differences in adaptive/maladaptive remodeling [62, 29].

The above considerations emphasize that the application of in vitro and transgenic results to human disease requires careful study and direct validation in human cardiac tissue samples.

## Direct Investigation of Sarcomere Function in Heart Samples from HCM Patients

In spite of many studies of mutations in experimental models of HCM, there have been few reports to date that actually directly document the functional abnormalities present in human HCM heart muscle. The most significant reasons for this are the limited availability of affected tissue and of suitable control human myocardium. An important advancement for the definition of the structural and functional changes present in human HCM tissue can be the creation of large banks of affected and control tissue through the collaboration of a number of centers for HCM studies. This will provide researchers access to cardiac tissue harvested from fully characterized patients undergoing cardiac surgery.

Cardiac muscle cell contraction and relaxation are governed by the interplay between two macromolecular systems: (1) membrane-bound $Ca^{2+}$ transport proteins, responsible for the $Ca^{2+}$ signal that switches contraction on and off and (2) sarcomere proteins, responsible for passive and active force generation and contraction regulation by $Ca^{2+}$. Together, these macromolecular systems determine the extent and kinetics of cardiac cell contraction and relaxation and both expend large amounts of energy. To separate their roles in muscle function, skinned cardiac muscle preparations are employed. In these preparations, cell and sarcoplasmic reticulum membranes (and thus all $Ca^{2+}$ handling systems) are removed, allowing investigators to experimentally control [$Ca^{2+}$] and focus on the process of force generation and relaxation at the sarcomere level.

Skinned myocardial strips isolated from human hearts are the conventional preparations used to measure changes in fundamental indices of muscle performance accompanying alterations of human cardiac sarcomeric proteins (e.g., [18, 40, 42, 43]). These preparations, however, have important limitations. Because cardiac strips are cut from bulk muscle and myocytes are not perfectly aligned along the axis of the strip, these preparations may include regions of muscle which lack mechanical continuity. In addition, the fractional volume occupied by sarcomeres in cardiac strips may vary depending on the tissue samples from which they are isolated. These factors can be a large source of variability in mechanical parameters and significantly complicate the interpretation of the functional effects of sarcomeric protein mutations.

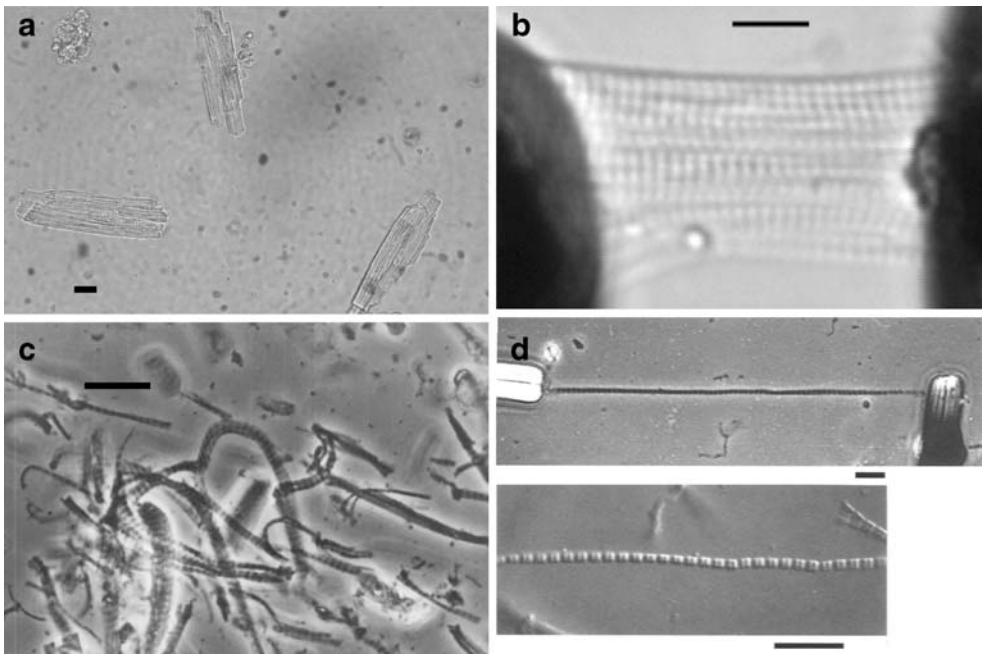


**Fig. 2** Skinned cardiac myocytes (**a** and **b**) and isolated cardiac myofibrils (**c** and **d**). *Calibration bars* in all panels are 10 μm. These preparations can be obtained in large amounts from small fresh or frozen human cardiac samples (see myocyte and myofibril suspensions in **a** and **c,** respectively) and can be used for mechanical experiments after mounting in force recording apparatuses (**b** and **d**). In **b,** a single human ventricular myocyte is glued between a force transducer and a piezo-electric motor (courtesy Dr. Jolanda van der Velden). In **d**, *upper panel*, a single myofibril is mounted between a cantilever force transducer and a glass microtool connected to a length-control motor. As better shown in the *bottom panel*, myofibrils are formed of single rows of serially arranged sarcomeres

Mechanically isolated skinned myocytes [71, 67] and single myofibrils [46] are a significant advance in the ability to directly measure changes in the mechanical performance of human cardiac muscle with altered sarcomeric proteins. These preparations can be obtained in significant amounts even from small fresh or frozen cardiac samples (Fig. 2) and enable direct correlations between in vivo hemodynamic patient characteristics and functional properties of the sarcomere [9].

Recent force measurements in mechanically disrupted Triton-permeabilized cardiomyocytes from HCM patients carrying truncation mutations in cMyBP-C show, compared with controls, a decrease in maximal isometric force per cross-sectional area and an increase in myofilament $Ca^{2+}$-sensitivity [68, 25]. These changes are accompanied by reduction (25–35%) in myofibril cMyBP-C content, a direct consequence of the truncation mutations. Though the increased $Ca^{2+}$-sensitivity may be a secondary consequence of altered phosphorylation levels of the sarcomeric proteins [68], previous functional studies, in which cMyBP-C has been partially extracted from skinned animal cardiomyocytes, indicate that the observed reduction in protein content in human HCM myocytes may be sufficient to cause both the observed mechanical changes [22, 30].

Isolated cardiac myofibrils, as a model for mechanical kinetics experiments, hold some advantage over larger preparations [47, 59]. Single myofibrils are the smallest units of the contractile apparatus of striated muscle that retain the organized myofilament lattice and entire ensemble of associated proteins. Miniaturized mechanical methods measuring myofibril force have been developed in the past years. Because of their small size (the diffusion distances are < 2 μm), single myofibrils equilibrate with bathing solutions in less than 1 ms, and the myofilament lattice can be effectively clamped at any desired composition by flowing streams of solution over the preparation. Rapid solution switching (Fig. 3a), originally developed for other biological applications [54], can thus be used in isolated myofibrils (1) to abruptly change the concentration of $Ca^{2+}$ and investigate force activation and relaxation kinetics ([64], see Fig. 3b), (2) to abruptly change the concentrations of substrate (MgATP) and products (Pi and MgADP) of the acto-myosin ATPase and dissect some specific steps of the crossbridge chemomechanical cycle [63].

A recent myofibril study [7] is the first to directly examine the mechanical kinetics of cardiac sarcomeres from a patient affected by HCM. The patient carried the severe R403Q mutation in the β-MyHC. In the R403Q myofibrils, maximal isometric tension was lower—or tended to be lower—relative to control groups, whereas the kinetics of tension development and relaxation were faster (Fig. 4), indicative of faster crossbridge turnover rate, an apparent (but probably not real) gain of protein function. The observation that the rate constant of the initial, slow, isometric phase of relaxation (slow $k_{REL}$) markedly increased in the R403Q myofibrils compared with controls is rather striking and shows that the mutation increases the

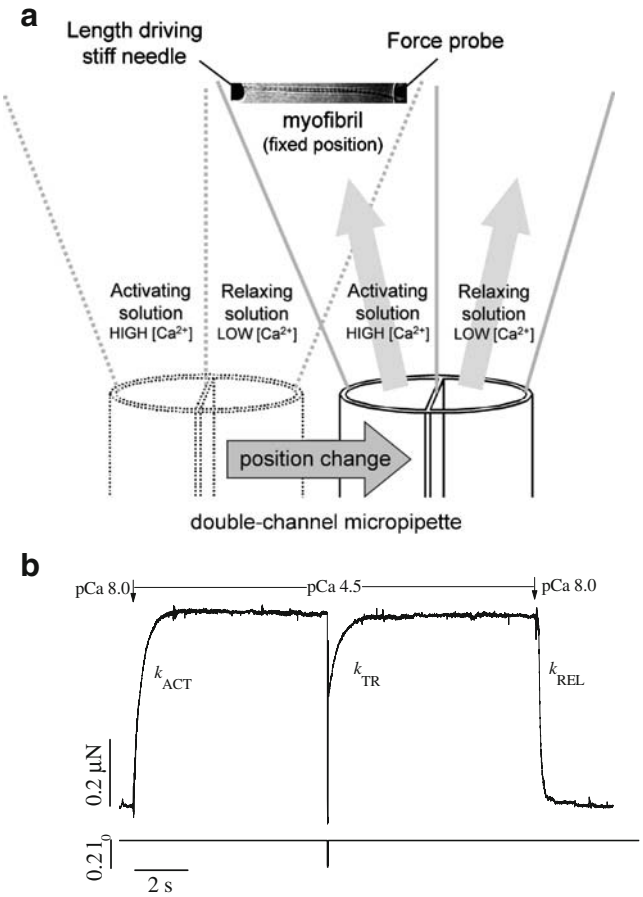


**Fig. 3** Fast solution-switching to measure force activation and relaxation kinetics in human cardiac myofibrils. **a** Experimental setup. The myofibril is mounted horizontally between two glass microtools. One tool is connected to a length-control motor that can produce rapid (<1 ms) length changes. The second tool is a calibrated cantilevered force probe. Force is measured from the deflection of the image of the force probe projected on a split photodiode. Myofibrils are maximally activated and fully relaxed by rapid translation between two continuous streams of relaxing (pCa=$-\log_{10}$ [$Ca^{2+}$]=8.0) and activating (pCa 4.5) solutions flowing from a double-barreled glass pipette placed at a right angle to the preparation. Solution change after the start of the pipette movement is complete within 10 ms [64]. To induce $Ca^{2+}$-activated force development, the pipette is switched from the left position (flow configuration drawn in *dashed lines*) to the right (*solid lines*). To induce relaxation, the movement is reversed. Modified from [59]. **b** Representative force response (*top trace*) of a human atrial myofibril maximally activated and fully relaxed by fast solution-switching (pCa changes at *arrows* as indicated). Initial sarcomere length 2.2 μm, temperature 15°C. Fast length changes (*bottom trace*) are applied to the myofibril under conditions of steady tension generation. $k_{ACT}$ is the rate constant of tension generation following fast $Ca^{2+}$ activation; $k_{TR}$ is the rate constant of tension redevelopment following the rapid length perturbation; $k_{REL}$ are the rate constants of the biphasic tension relaxation following fast $Ca^{2+}$ removal (see Fig. 4e)

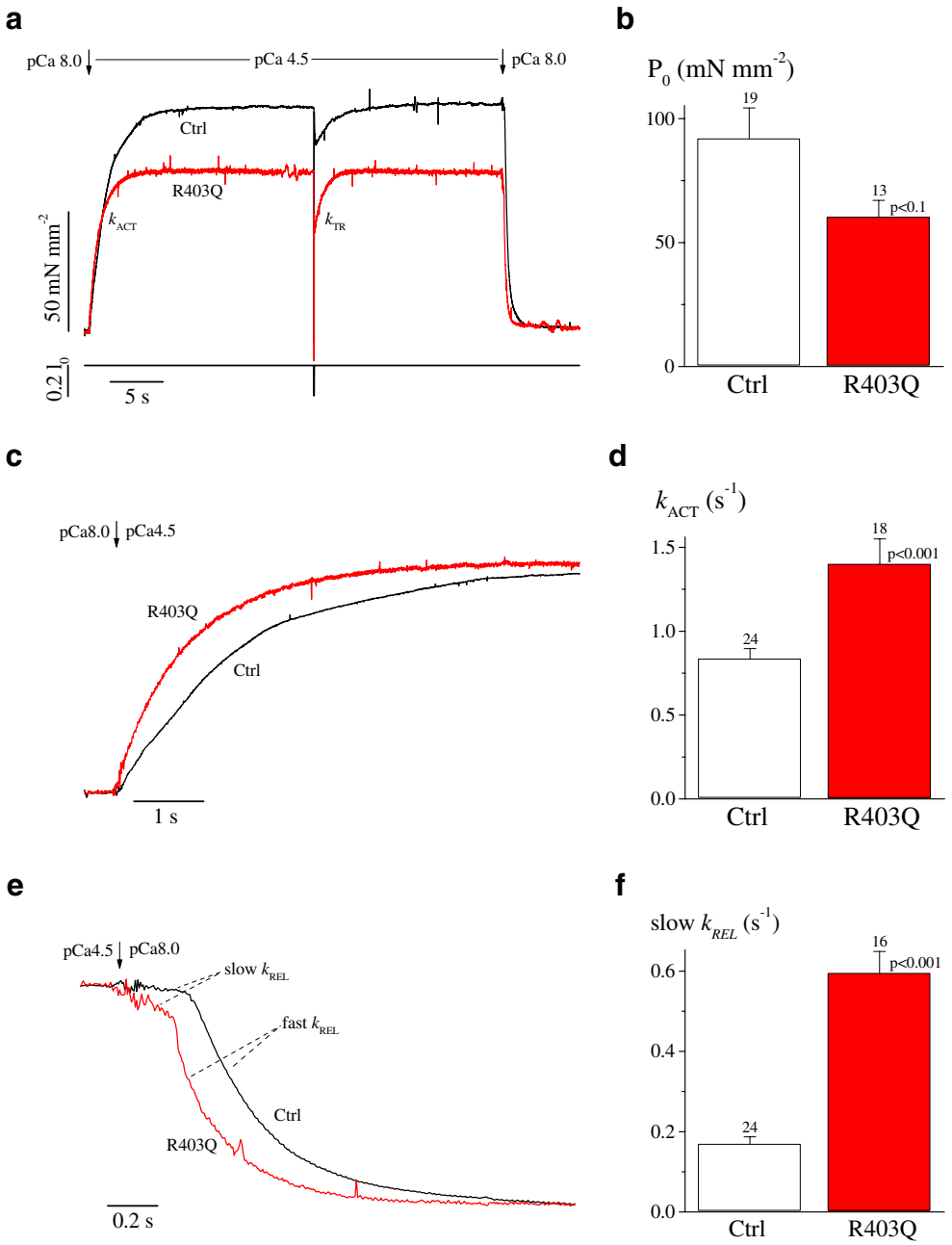


**Fig. 4** Tension generation and relaxation in control donor and R403Q left ventricular myofibrils. Data from [7] **a** representative tension responses (*top traces*) of donor and R403Q myofibrils maximally activated and fully relaxed by fast solution-switching (pCa changes at *arrows* as indicated). Temperature 15°C. Fast length changes (*bottom traces*) are applied to the myofibrils under conditions of steady tension generation. **b** Mean values of maximal tension ($P_0$) for the donor (*white column*) and R403Q (*red column*) myofibrils. *Bars* on *top of columns* are SEM; the number of myofibrils is given on top of SEM bars. **c** The time course of tension activation following sudden [$Ca^{2+}$] increase of the donor and R403Q myofibrils shown in **(a)** are superimposed on a faster time base after normalization for maximal tension. (**d**) Mean values of $k_{ACT}$ for the donor (*white column*) and R403Q (*red column*) myofibrils. *Bars* and *numbers* as in **(b)**. **e** Tension relaxation kinetics following sudden $Ca^{2+}$ removal; same traces as in **a** superimposed on a faster time base after normalization for maximal tension. The rate constant of the early slow-force decline (slow $k_{REL}$) is estimated from the slope of the regression line fitted to the tension trace normalized to the entire amplitude of the tension relaxation transient. The rate constant for the final fast phase of tension decline (fast $k_{REL}$) is estimated from mono-exponential fit. **f** Mean values of slow $k_{REL}$ for the donor (*white column*) and R403Q (*red column*) myofibrils. *Bars* and *numbers* as in **b**

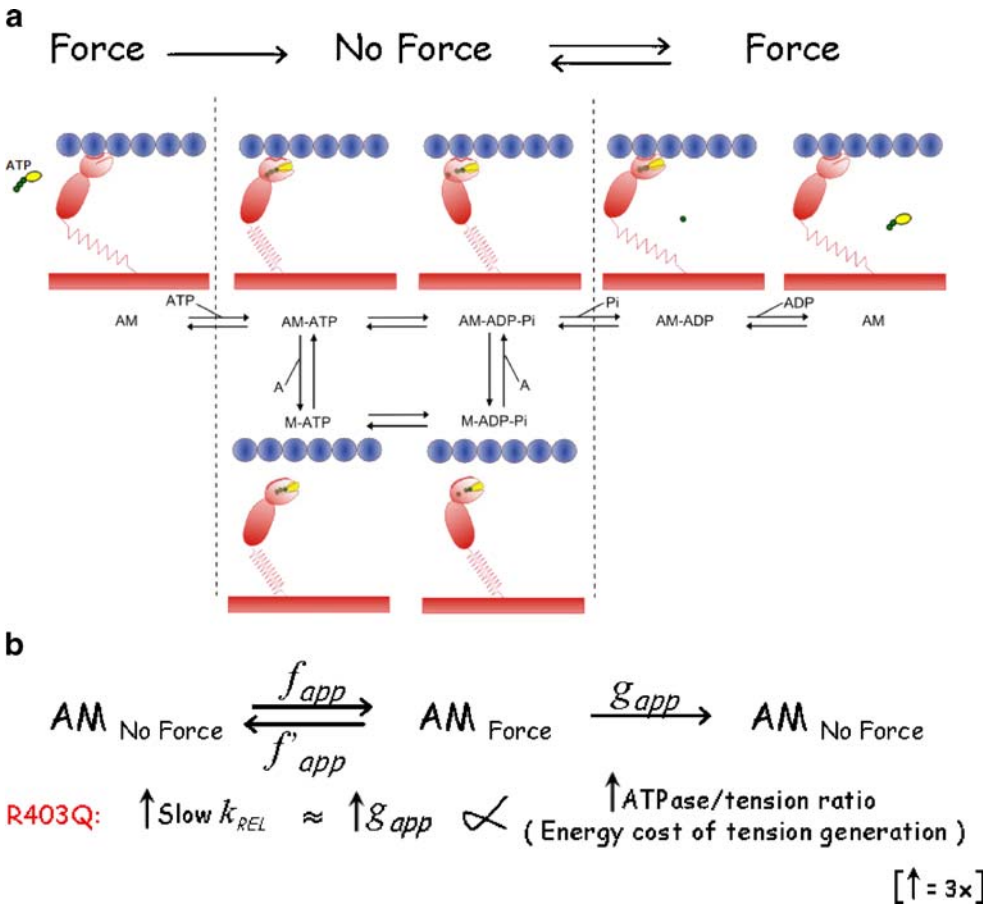


**Fig. 5** Reaction pathway for acto-myosin ATPase and energy transduction cycle. **a** The reaction pathway can be described as a series of coupled biochemical and mechanical events. The structure of the myosin head (depicted as in Fig. 1 with the light chain binding domain that can move like a lever arm relative to the actin-binding, catalytic domain) is designed to achieve the efficient conversion of biochemical energy. ATP binding to a myosin head (M) causes a rapid, almost irreversible dissociation of the myosin head from actin (A). Following detachment from actin, the ATP is hydrolyzed to ADP and inorganic phosphate ($P_i$) both of which remain tightly bound to the myosin head. The free energy of ATP hydrolysis is not released but remains within the structure of the M.ADP.$P_i$ complex. The hydrolysis is, in fact, accompanied by a major conformational change that represents a repriming of the power stroke; both the hydrolysis and the conformational change are reversible. ADP and $P_i$ will remain bound to the myosin head until the myosin binds to an actin site. The affinity of M.ADP.$P_i$ for actin is significantly higher than that of M.ATP. If an actin site is within reach of the myosin head, it will bind rapidly and reversibly to the actin site. The interaction of the M.ADP.$P_i$ complex with actin can promote a major change in conformation (the power stroke) which is accompanied by the dissociation of $P_i$. If the filaments carry an external load (e.g., isometric conditions), then the power stroke results in the distortion of an elastic element. The location of the elastic element is unknown and is drawn here, for simplicity, as part of the S2 region connecting the myosin head to the rod. The dissociation of $P_i$ is a reversible event and $P_i$ can rebind to reverse the power stroke. The final step of the chemo-mechanical coupling is a strain-dependent mechanism of ADP release leading to an AM complex rapidly dissociated by new ATP binding. ADP release can be very fast if the external load on the filaments is small while it is much slower under high loads (isometric conditions). The features of this transition likely differ between myosin designed for efficient fast shortening vs efficient load bearing. In this generally accepted scheme, transitions of crossbridges from force-generating to non-force-generating states involved in isometric force relaxation comprise both forward (ADP release and ATP binding) and backward ($P_i$ rebinding and reversal of the power stroke) steps. However, myofibril slow (isometric) $k_{REL}$, measured in the absence of $P_i$ (as in the case of [7]), only probes forward detachment transitions that lead to ATP hydrolysis steps. **b** 2-state crossbridge scheme. On the basis of myosin's binding affinity for actin, two general categories of cross-bridges can be defined: $AM_{no\ force}$ represents all weak binding states (detached states M.ATP, M.ATP.$P_i$ in rapid equilibrium with AM.ATP, AM.ADP.$P_i$) and $AM_{force}$ all strong binding states (AM, AM.ADP). The transition from the non-force-generating states to the force-generating states has an apparent rate constant $f_{app}$, whereas $g_{app}$ describes the return to the non-force-generating states by means of ADP release and ATP binding. The apparent rate constant for the reverse transition $f'_{app}$ that depends on [$P_i$] can be neglected under the conditions of the study by [7] (absence of $P_i$) and myofibril slow $k_{REL} \approx g_{app}$. In the model, the energy cost of tension generation (ATPase/tension ratio) is proportional to $g_{app}$. Given slow $k_{REL} \approx g_{app}$, the increase in slow $k_{REL}$ associated with the R403Q mutation predicts a 3-fold increase in the energy cost of tension generation in R403Q sarcomeres [7]

apparent rate of dissociation of myosin from actin under isometric conditions ($g_{app}$; see Fig. 5). Analysis with simple crossbridge models of sarcomere force generation indicates that the increase in $g_{app}$ associated with the R403Q mutation accounts almost entirely for the faster isometric crossbridge turnover (faster rate of force generation) and the lower maximal isometric tension. The marked increase in $g_{app}$ under isometric conditions is expected to greatly increase the overall tension cost in the HCM heart (Fig. 5b) consistent with a wide range of biochemical [27] and NMR-based studies in both HCM patients [11] and animal models [58, 26, 19] of the disease. Though only direct measurements of ATPase activity under isometric conditions (see [43] for possible methods) can definitely confirm this conclusion, the results strongly support the idea that the R403Q mutation leads to inefficient ATP utilization for tension generation.

The finding that relaxation kinetics are faster in the R403Q myofibrils than in the controls is apparently inconsistent with clinical evidence of diastolic dysfunction associated with HCM [20]. In the whole heart, however, multiple mechanisms determine the time course of tension relaxation while in the myofibril experiments we focus solely on the sarcomeric determinants of the relaxation process independent of disease-associated secondary changes occurring in other cell functions and sarcomere ionic microenvironment. For example, in intact cardiac myocytes, the mechanisms related to $[Ca^{2+}]$ reduction in the myoplasm are important determinants of the time course of force relaxation. In the intact myocardium, compromised energetics may slow down relaxation by altering the $Ca^{2+}$ transport function needed for $Ca^{2+}$ removal from the myoplasm. Compromised energetics may also alter the ionic microenvironment of contractile proteins and this, in turn, can impair force relaxation.

Myofibril studies on HCM patients can represent an important advance both with respect to conclusions about specific mutations at the level of the cardiac crossbridge cycle and its regulation and, as a methodology, to provide a unique and more biophysically proximal phenotypic characterization of HCM.

## Conclusions

Though direct analysis of the altered functional characteristics of HCM human cardiac sarcomeres has so far lagged behind the in vitro and mouse studies, initial results seem to validate current pathogenetic hypotheses based on a number of studies in experimental HCM models [3]. It is encouraging that these hypotheses implicate targets that can be much more amenable to pharmacological alteration than might have been expected from the initial finding of structural alteration of the myofilament proteins. For example, many existing agents have been developed for amelioration of myocardial energetics (typically, in the context of ischemic heart disease), e.g., by switching substrate utilization. Experimental evidence in rodent models suggests that agents, to decrease myofilament calcium sensitivity (not yet available in man), could also be expected to have markedly beneficial effects particularly on propensity to arrhythmia [4].

**Acknowledgements** Financial support by Telethon-Italy (grant # GGP07133), Ente Cassa di Risparmio di Firenze, and European Union (FP7-HEALTH-2009-single-stage, BIG-HEART 241577) is gratefully acknowledged.

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