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Hereditary heart disease: pathophysiology, clinical presentation, and animal models of HCM, RCM and DCM associated with mutations in cardiac myosin light chains

Sunil Yadav^{1,✱}, Yoel H. Sitbon^{1,✱}, Katarzyna Kazmierczak¹, and Danuta Szczesna-Cordary^{1,*}

¹Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, Miami, FL 33136

Abstract

Genetic cardiomyopathies, a group of cardiovascular disorders based on ventricular morphology and function, are amongst the leading causes of morbidity and mortality worldwide. Such genetically-driven forms of hypertrophic (HCM), dilated (DCM) and restrictive (RCM) cardiomyopathies are chronic, debilitating diseases that result from biomechanical defects in cardiac muscle contraction and frequently progress to heart failure (HF). Locus and allelic heterogeneity, as well as clinical variability combined with genetic and phenotypic overlap between different cardiomyopathies, have challenged proper clinical prognosis and provided an incentive for identification of pathogenic variants. This review attempts to provide an overview of inherited cardiomyopathies with a focus on their genetic etiology in myosin regulatory (RLC) and essential (ELC) light chains, which are EF-hand protein family members with important structural and regulatory roles. From the clinical discovery of cardiomyopathy-linked light chain mutations in patients to an array of exploratory studies in animals, reconstituted, and recombinant systems, we have summarized the current state of knowledge on light chain mutations and how they induce physiological disease states via biochemical and biomechanical alterations at the molecular, tissue and organ level. Cardiac myosin RLC phosphorylation and the N-terminus ELC have been discussed as two important emerging modalities with important implications in the regulation of myosin motor function and thus cardiac performance. A comprehensive understanding of such triggers is absolutely necessary for the development of target-specific rescue strategies to ameliorate or reverse the effects of myosin light chain-related inherited cardiomyopathies.

Keywords

Cardiomyopathy mutations; myosin regulatory light chain; myosin essential light chain; human phenotype; transgenic mice

*Address correspondence to: Danuta Szczesna-Cordary, University of Miami Miller School of Medicine, 1600 NW 10th Ave., RMSB 6113, Miami FL 33136. Phone: 305-2432908, Fax: 305-2434555, dszczesna@miami.edu.

✱These authors contributed equally

Introduction

Mutations in genes encoding sarcomeric proteins are the most predominant causes of inherited cardiomyopathies. In this review, we report on mutations in myosin regulatory (RLC) and essential (ELC) light chains that are associated with one or more types of cardiomyopathy disease: hypertrophic (HCM), dilated (DCM) and restrictive (RCM) cardiomyopathy ⁷⁶ (Table 1).

HCM is a disease of the myocardium characterized by asymmetric hypertrophy of the left ventricle (LV) and impaired diastolic function^{76, 104}. The prevalence of HCM is 1 in 500 of the general population, and occurrences of sudden cardiac death (SCD) are common, especially among young athletes ⁷⁵. Histopathological features of HCM include myocyte enlargement (hypertrophy) and myofibrillar disarray, changes which are often accompanied by fibrotic depositions ^{31, 73}. Dominant pathogenic variants in HCM genes are the most common causes of HCM ³, and the two predominant genes responsible for approximately half of the patients with familial HCM are *MYH7* (β -myosin heavy chain) and *MYBPC3* (myosin binding protein C) ⁷³. Mutations in *MYL2* or *MYL3*, encoding the ventricular myosin RLC or ELC, respectively, are rare, but they are often implicated in malignant HCM outcomes ^{26, 54, 84, 92}

In DCM, enlargement of the LV cavity (without a proportional increase of wall thickness) is observed which is followed by systolic dysfunction. Histopathological images of DCM hearts show vacuolated myocytes with myofibrillar loss and interstitial fibrosis ^{51, 90}. The prevalence of DCM in the general population is 1 in 250 ^{19, 43}. The majority of familial DCM is caused by genetic mutations with an autosomal dominant trait ⁷⁸ and 35-40% of these mutations are linked to sarcomeric proteins ⁴¹.

RCM is characterized by increased stiffness of the LV wall with no increase in wall thickness and a largely impaired diastolic function with usually normal or near-normal systolic function ⁶³. RCM is less common than HCM and DCM, but the prognosis is poor and the risk for ischemia-related complications and death is high ⁹⁶. The restrictive phenotype can also be observed in HCM families^{63, 89}. The majority of idiopathic RCM is caused by genetic mutations identified in myosin heavy chain, myosin binding protein C, troponin I and T, tropomyosin and desmin genes ²⁹.

The role of MLCs in cardiac muscle contraction

Cardiac muscle contraction involves the ATP-dependent cyclic attachments and detachments of the myosin cross-bridges to the actin-tropomyosin (Tm)-troponin (Tn) filaments ^{31, 66}. The myosin cross-bridge is the molecular motor of the heart; it binds ATP and actin and its neck region (lever arm) amplifies small conformational changes generated in the motor domain into large movements needed to produce force and sarcomere shortening ⁴⁴. Myosin head domain (S1) contains the ATP and actin binding sites and the lever arm domain where MLCs are attached to their respective IQ motifs⁹⁴. The myosin regulatory (RLC) and essential (ELC) light chains structurally support the lever arm region of myosin heavy chain (MHC) (Fig. 1)², but they also modulate myosin motor activity and thus cardiac muscle

contraction in light chain-dependent manner (review in ^{42, 109}). Mutations in ventricular myosin heavy chain (MHC) and both MLCs have been associated with different forms of cardiomyopathy ^{2, 20, 32, 35, 121} and over the last two decades, our lab has generated humanized mouse models of RLC and ELC related HCM/RCM/DCM ^{59, 60, 85, 111, 132-134} expressing the wild-type/mutant human cardiac isoforms of RLC/ELC.

Striated muscle myosin can be found in three states: an active/force-producing state, a disordered relaxed (DRX) state, in which myosin heads protrude into the interfilament space but are restricted from binding to actin, and a super-relaxed (SRX) state characterized by an ordered head arrangement along the thick filament axis and a highly inhibited ATP turnover rate ^{45, 79, 107}. The presence of the folded state of myosin, where the heads interact with each other and with myosin tail (S2) is hypothesized to be the origin of SRX ^{7, 120}. The cardiac SRX serves as a modulator of cardiac energy utilization, involved in decreasing metabolic rate (load) in both, the normally functioning myocardium and during times of stress, e.g. cardiomyopathy ^{45, 79}. The important question in the area of myosin RLC and ELC research regards the role of MLCs disease-causing mutations in modifying the super-relaxed state of myosin and controlling the SRX ↔ DRX equilibrium ^{7, 106}, as well as contributing to the regulation of myosin's power stroke, ATP utilization and force production in muscle.

Myosin RLC (*MYL2*)

There are two unique features of myosin RLC: the Mg²⁺ and Ca²⁺ binding site, containing a helix-loop-helix motif (EF1), and the myosin light chain kinase (MLCK) dependent phosphorylation site, both located in the N-terminus of RLC ¹⁰⁹ (Fig. 2). Under physiological conditions, in relaxed muscle, it is thought that this site is occupied by Mg²⁺ and may become partially saturated with Ca²⁺, depending on the length of the [Ca²⁺] transient ^{97, 115}. In rodent cardiac RLC, there are two phosphorylation sites, at serine 14 and 15, which can be phosphorylated with cardiac MLCK (cMLCK), but also with zipper kinase (ZIP kinase) ¹⁰². Human cardiac RLC has only one phosphorylatable serine 15, but there is also adjacent asparagine 14 that can be deamidated, and both posttranslational modifications increase the negative charge of the RLC ¹⁰³ (Fig. 2).

MYL2 mutations

In 1996, initial RLC exon screening in HCM patients revealed the first three distinct mutations (A13T, E22K, P95A-initially reported as P95R), with A13T and E22K patients showing a particular type of familial HCM with pronounced mid-cavity obstruction (MVC) ⁹². A later report found the A13T-mutated patients to suffer from profound septal hypertrophy, exertion induced dyspnea and severe cardiac abnormalities, including left ventricular outflow tract (LVOT), which were enhanced by ergonomic exercise ⁵.

The **Ala13Thr (A13T)** mutation is located in the N-terminus of the myosin RLC (Fig. 2), which together with the ELC support the neck region of the myosin head attaching to their respective IQ motifs. Recombinant A13T RLC mutant resulted in a large increase in the α-helical content which decreased upon the binding of Ca²⁺ to A13T mutant and even returned to the level of wild-type (WT) RLC upon phosphorylation. Using flow dialysis, the group

showed a decrease in Ca^{2+} binding affinity compared with human cardiac RLC-WT. The study also showed that phosphorylation of A13T resulted in a large increase (increase in K_{Ca} value) in its Ca^{2+} binding affinity compared with non-phosphorylated A13T. A decrease in calcium sensitivity in rat ventricular RLC-reconstituted skinned rabbit psoas muscle fibers, without any change in maximal tension, was also reported by Roopnarine et al. ⁹⁸.

Transgenic mice expressing human ventricular A13T RLC mutation showed enlarged interventricular septa and profound fibrotic lesions. Cardiac muscle preparations from these mice revealed a 1.4-fold increase in maximal contractile force and ~25% decrease in actin-activated myosin ATPase activity compared to Tg-WT or NTg mice ⁵⁸. In that study, A13T was also shown to have lower binding affinity for RLC-depleted porcine myosin. It was hypothesized that the A13T mutation affects the myosin power stroke generation by changing the kinetic properties of the cross-bridges, possibly exerting a dominant-negative effect on cardiomyocyte structure and function. A13T mutation differs from other HCM-RLC mutations in that no changes in force/ATPase-pCa relationship as well as endogenous RLC phosphorylation in Tg mice myocardium were observed in the study ⁵⁸. A13T and F18L also resulted in dramatic reduction in unloaded shortening velocity at saturating myosin surface densities, suggesting that the amino acids around the phosphorylatable serine 15, play an important role in the myosin lever arm stabilization²⁵.

Soon after Poetter's discovery, two novel missense mutations, **Phe18Leu (F18L)** in exon 2 and **Arg58Gln (R58Q)** in exon 4 (Fig. 2) were identified in three unrelated families with a typical form of HCM, with increased left ventricular wall thickness and abnormal echocardiography with no mid-left ventricular obstruction ^{26, 95}. The mutated F18L residue is located in a protein domain in which two previously identified mutations (A13T and E22K) were shown to be responsible for the HCM phenotype ⁹². F18L was also shown to decrease calcium affinity of RLC compared with human cardiac RLC-WT and no effect of phosphorylation was observed on calcium binding. Ca^{2+} binding to F18L produced a slight increase in the α -helix but no change in α -helical content was observed for phosphorylated F18L-RLC¹⁰⁹. A slight decrease in calcium sensitivity of myofibrillar ATPase was shown in reconstituted porcine cardiac myofibrils ¹¹⁰ F18L was found to decrease tension, Ca^{2+} sensitivity, and cooperativity in reconstituted rat ventricular RLC system ⁹⁸.

Met20Leu (M20L) (along with E134A and G162R) (Fig. 2) was identified as a novel mutation in a cohort of 203 unrelated patients with HCM in a study to determine the influence of a positive genetic test for HCM on clinical outcome ⁸⁸. Single molecule measurements in mutant RLC reconstituted porcine cardiac fibers by Burghardt et al. found M20L fibers to produce maximal isometric force with below normal lever-arm stiffness ¹². Using TIRF, lever-arm orientation was measured in rigor, isometric contraction, and relaxation. It was noted that radical reconfiguration in M20L reinforces the suggestion that light chain modification near the RLC phosphorylation site at Ser15 affects function, linking the M20L phenotype to lower efficiency for ATP free-energy conversion to mechanical work ¹².

In addition to Poetter's 1996 discovery of **Glu22Lys (E22K)** (Fig. 2) in three patients from two unrelated families with MVC phenotype, the mutation was later associated with moderate septal hypertrophy, late onset of clinical manifestation, and benign disease course

and prognosis⁵⁴. Similarly, this mutation was also reported homozygously in two Spanish patients by Garcia-Pavia et al. during the genetic screening of a cohort of 26 patients, transplanted for end-stage HCM³⁰. In addition, Claes et al. reported E22K mutation in 14 apparently unrelated HCM families and suggested that the mutation, on its own, was incapable of triggering clinical HCM and might require the presence of an additional risk factor for hypertrophy, particularly hypertension¹⁸. Interestingly, E22K mouse model from Robbins group poorly recapitulated *in vivo* alterations found in human as transgenic mice carrying E22K RLC failed to exhibit either overt hypertrophy or mid-ventricular cavity obstruction despite changes at the myofilament and cellular levels, with the myofibrils showing increased Ca^{2+} sensitivity and significant deficits in relaxation in a transgene dose-dependent manner⁹⁹. Similarly, echocardiography abnormalities seen in most of the human patients with the E22K mutation were not detected in transgenic mice expressing mutant human ventricular E22K RLC, despite visibly enlarged inter-ventricular septa as well as papillary muscles seen in H&E staining¹¹¹. Skinned cardiac muscle preparation showed an increase in calcium sensitivity of myofibrillar ATPase activity and force development, leading to the idea that E22K-mediated HCM may be mediated via Ca^{2+} -dependent levels¹¹¹. Likewise, E22K was found to increase Ca^{2+} sensitivity of force in reconstituted rat ventricular RLC system⁹⁸. Such increase in calcium sensitivity of force development was also shown in slow skeletal muscle fibers, containing same RLC isoform as that of cardiac, from biopsy samples from patients with inherited HCM carrying the E22K RLC mutation⁶⁵. In *in vitro* studies, E22K mutation was shown to render the protein non-phosphorylatable, increase α -helical content, decrease calcium binding affinity (by ~20 fold) to isolated RLC¹¹⁴. No effect of the E22K mutation on the kinetics of mutated myosin cross-bridges in its ATP-powered interaction with fluorescently labeled single actin filaments was observed by Dumka et al.²². However, maximal force and ATPase were found to be ~20% decreased in Tg-E22K skinned papillary muscle fibers from mice compared with Tg-WT animals¹¹². In addition, intracellular $[\text{Ca}^{2+}]$ and force transients were significantly faster in intact papillary muscle fibers from Tg-E22K compared to Tg-WT cardiac mice preparations¹¹².

Santos et al. identified a novel **Ile44Met (I44M)** *MYL2* mutation (Fig. 2) in Portuguese population presenting with familial history of HCM or clinical diagnosis using High Resolution Melting technology by means of analyzing 28 HCM-associated genes, including the most frequent 4 HCM-associated sarcomere genes, as well as 24 genes with lower reported HCM-phenotype association¹⁰⁰. further experimental studies are needed to investigate the potential mechanisms for I44M-induced HCM.

The **Asn47Lys (N47K)** mutation in *MYL2* (Fig. 2) was discovered during screening of 68 Danish and 130 South African HCM patients and was found to be associated with a mid-ventricular hypertrophy (MVH) phenotype in a Danish patient, found to cause a late onset of the disease with a rapidly progressing MVH phenotype and a significant increase in the size of the papillary muscles, but with an absence of SCD⁵. Previous studies have determined the N47K-mediated depression of cardiac function in transgenic mice and in β -MHC reconstituted porcine cardiac muscle preparations, without affecting the MLCK-dependent phosphorylation of RLC in the ventricles of Tg mice^{1, 36, 37, 55, 124}. N47K substitution in the second from last coordinating position of the Ca^{2+} binding loop of human cardiac RLC

was shown to abolish its Ca^{2+} binding and increase the Ca^{2+} sensitivity of myofibrillar ATPase, without any change in the calcium sensitivity of force¹¹⁰. N47K both showed a reduction in isometric force with an increase in duty cycle, meaning that the attachment time would be longer, increasing the probability of “dragging heads”³⁶. The N47K mutation also caused a reduction in isometric force compared with native and wild-type myosin and appeared to cause a reduction in the peak power output as well as the load at which peak power occurs³⁷. In addition, N47K mutation resulted in prolonged $[\text{Ca}^{2+}]$ transients assessed in intact papillary muscle fibers with no change in calcium sensitivity of force or ATPase in transgenic N47K muscle fibers¹²⁴. Cardiac output, cardiac work, and cardiac power were significantly decreased in Tg-N47K aerobically perfused and spontaneously beating hearts compared with controls¹. N47K mutant showed slower cross-bridge cycles, higher actin-activated ATPase activity and exhibited larger tension than WT; however, no changes in rigor actomyosin binding were observed and a significant decrease in $\text{dP/dt}_{\text{max}}$ -EDV relationship, indicative of depressed ventricular contractility in N47K mice was reported¹²³.

Caleshu et al. discovered another point mutation, **Gly57Glu (G57E)** (Fig. 2), in *MYL2* along with previously reported homozygous E143K ELC mutation in a 22-year-old patient with restrictive cardiomyopathy (RCM) presenting with class III/IV heart failure and requiring heart transplantation. Her mother was a double heterozygote for these mutations, with no evidence of cardiomyopathy¹⁶.

Single-strand polymorphism analysis of exon 4 in two unrelated probands of families revealed the **Arg58Gln (R58Q)** mutation (Fig. 2), responsible for “classic” HCM phenotype with no mid-ventricular hypertrophy²⁶. The R58Q mutation showed moderate septal hypertrophy, but in contrast, it was associated with an early onset of clinical manifestation and premature sudden cardiac death⁵⁴. The R58Q mutant did not bind Ca^{2+} and interestingly, Ca^{2+} binding to the R58Q-RLC was restored upon phosphorylation. In addition, the α -helical content of R58Q was greatly increased upon calcium binding¹¹⁴. No change in α -helical content was observed for the phosphorylated R58Q in the apo-state¹¹⁴. Echocardiographic evaluations of transgenic R58Q mice showed significant alterations in diastolic transmitral velocities and deceleration time and isolated myofibrils exhibited changes in Ca^{2+} sensitivity, cooperativity, and an increased level of ATPase activity at low $[\text{Ca}^{2+}]$ along with reduced endogenous RLC phosphorylation in Tg ventricles¹. R58Q mutation exhibited much more severe changes in cardiac function, including more reduced cardiac work as well as cardiac power compared with control hearts¹. Simultaneous measurements of the ATPase and force in skinned papillary muscle fibers obtained from transgenic R58Q mice showed an increase in the Ca^{2+} sensitivity of ATPase and steady-state force as well as manifested prolonged force and $[\text{Ca}^{2+}]$ transients in electrically stimulated intact papillary muscles¹²⁴. A significant increase in ATPase was observed for R58Q myosin that was hypothesized to offset part of the increase in observed duty cycle, thereby imparting R58Q an increased sliding velocity in the *in vitro* motility assays on myosin isolated from transgenic mice³⁶. R58Q was also shown to reduce isometric force compared to the WT which could lead to compensatory hypertrophy. Consistent with this, fiber studies of the R58Q mutation done previously in skinned muscle fibers also showed a shift towards

submaximal calcium activation of ATPase compared to WT¹²⁴. In addition, R58Q-exchanged myosins show reductions in force and power output compared with WT or native myosin and the changes in loaded kinetics were thought to result from mutation-induced loss of myosin strain sensitivity of ADP affinity³⁷. Yadav et al., using a phosphomimic recombinant RLC variant where the phosphorylation site Ser-15 was substituted with aspartic acid (S15D) and placed in the background of R58Q, showed a multitude of rescue effects of R58Q-exerted adverse phenotypes in S15D-R58Q-reconstituted porcine cardiac muscle preparations¹³¹. A low level of maximal isometric force observed for R58Q- vs. WT-reconstituted fibers was restored by S15D-R58Q. The authors also report that R58Q promotes the OFF state of myosin, both in reconstituted porcine fibers and in transgenic mouse papillary muscles, thereby stabilizing the super-relaxed state (SRX) of myosin, characterized by a very low ATP turnover rate¹³¹. Experiments in S15D-R58Q-reconstituted porcine fibers showed a mild destabilization of the SRX state, suggesting an S15D-mediated shift in disordered-relaxed (DRX) ↔ SRX equilibrium towards the DRX state of myosin. The study concluded that S15D-phosphomimic can be used as a potential rescue strategy to abrogate/alleviate the RLC mutation-induced phenotypes¹³¹. Interestingly, it was recently shown that R58Q-mutant mice showed functional similarities between the cardiac papillary muscles and slow-twitch soleus skeletal muscle, as evident by lower contractile force in both papillary and soleus muscles compared to fast-twitch extensor digitorum longus muscles of R58Q vs. wild-type–RLC mice⁶¹. This coincided a decreased proportion of fiber type I/type II only in SOL muscles but not in the extensor digitorum longus muscles, as well as in differential regulation of proteins between the heart and SOL muscles of R58Q mice. These data suggest that *MYL2* models of HCM may be used as a model to study the molecular, structural, and energetic mechanisms of RLC mutation-induced cardioskeletal myopathy⁶¹.

Huang et al. described a novel **Asp94Ala (D94A)** DCM mutation in *MYL2* (Fig. 2), in which aspartic acid at position 94 is replaced by alanine (D94A)⁴⁹. It was identified by Dr. Hershberger group using exome sequencing in a pedigree with familial DCM. Recombinant D94A protein induced a reduction in the α-helical content of the RLC, without any change in its ability to be phosphorylated by Ca²⁺/calmodulin-activated MLCK⁴⁹. The mutation was shown to impair binding to the myosin heavy chain and had significantly higher actin-activated ATPase activity. D94A reconstituted porcine papillary muscles exhibited slightly reduced maximal tension with no change in the calcium sensitivity of force⁴⁹. Transgenic D94A mice, generated by Yuan et al.¹³⁴ exhibited a significant reduction in the ejection fraction, with younger male D94A mice showing a more pronounced LV chamber dilation compared with female counterparts. Papillary muscles isolated from transgenic D94A mice showed a rightward shift of the force–pCa dependence and decreased actin-activated myosin ATPase activity. Small-angle X-ray diffraction study at submaximal Ca²⁺ concentrations revealed repositioning of cross-bridge mass toward the thick-filament backbone, supporting the hypocontractile state of D94A myosin motors¹³⁴.

Pro95Ala (P95A) (originally mislabeled as P95R) was identified along with A13T and E22K, with pronounced mid-ventricular obstruction⁹². Flow dialysis experiments with recombinant P95A revealed a decrease in calcium binding affinity, with an increase in the α-helical content upon subsequent calcium binding¹¹⁴. P95A-reconstituted porcine cardiac

myofibrils showed the lowest ATPase activity with no change in calcium sensitivity of myofibrillar ATPase activity¹¹⁰. Experiments in reconstituted skinned rabbit psoas muscle fibers showed no change in maximal tension, calcium sensitivity of force, or cooperativity of activation of force generation⁹⁸.

Lys104Glu (K104E) and **IVS6-1** were initially identified in an *MYL2* mutational screening that identified SSCP conformers in both exon 5 and exon 7 (Fig. 2) in the Danish proband presenting with pronounced proximal septal hypertrophy, and diastolic filling abnormality as evidenced by an inverted transmitral flow pattern⁵. Transgenic mice expressing the Lys104Glu mutation did not show major histopathological abnormalities or myofilament disarray, except for in older animals, but showed early signs of diastolic disturbance with significantly reduced E/A transmitral velocities ratio along with a borderline significant prolonged isovolumic relaxation time (Tau) and a tendency for slower rate of pressure decline as evident in invasive hemodynamics studies. K104E hearts also showed higher mitochondrial content which was supported by higher ATPase activity and skinned papillary muscles showed a reduction in maximal tension and slower muscle relaxation rates⁴⁸. Mutated cross-bridges were significantly better ordered during steady-state contraction and during rigor, but the mutation had no effect on the degree of order in relaxed myofibrils. The K104E mutation increased the rate of XB binding to thin filaments and the rate of execution of the power stroke. Stopped-flow experiments revealed a significantly faster dissociation rate observed in Tg-K104E vs. Tg-wild-type (WT) myosin and a smaller second-order ATP-binding rate for the K104E compared with WT myosin²¹. Barth et al. reported a new skeletal muscle fiber type-I myopathy in three unrelated Dutch families with progressive cardiomyopathy and early infant deaths due to cardiac failure¹¹, and the genetic cause was only recently identified by Wetermen et al. to be a homozygous mutation in the last acceptor splice site of the myosin regulatory light chain 2 gene which results in use of a cryptic splice site upstream of the last exon causing a frameshift and replacement of the last 32 codons by 20 different codons¹²⁹ (Fig. 2).

Recombinant **IVS6-1-RLC** showed decreased binding to the MHC while IVS6-1-reconstituted porcine myosin displayed reduced binding to actin in rigor and demonstrated a significantly lower $V_{\max}$ of the actin-activated myosin ATPase activity¹³⁵. IVS6-1-reconstituted myosin also showed slower kinetics of the ATP-induced dissociation of the acto-myosin complex and a significantly reduced slope of the $k_{\text{obs}}\text{-[MgATP]}$ relationship as observed by stopped-flow fast kinetic studies¹³⁵. In addition, a significantly decreased maximal contractile force and a significantly increased Ca^{2+} sensitivity, which are both hallmarks of HCM-associated mutations, were observed in skinned papillary muscles reconstituted with IVS6-1-RLC¹³⁵. DNA SSCP analysis of exon and flanking intronic regions, followed by sequencing of an abnormal pattern on a capillary DNA sequencer, identified a splice acceptor site mutation (IVS5-2) in the European population. One donor-site splice mutation (IVS5-1:A>G) was predicted to lead to a premature termination codon⁹⁵.

Olivotto et al. discovered novel missense *MYL2* mutations, **Glu134Ala (E134A)**, in Exon 6 and **Gly162Arg (G162R)** in Exon 7 (Fig. 2), while determining the influence of a positive genetic test for HCM on clinical outcome in a cohort of 203 unrelated patients with HCM.

They showed that patients with myofilament-positive HCM exhibited an increased risk of the combined end-points of cardiovascular death, nonfatal stroke, or progression to New York Heart Association class III or IV ⁸⁸. Burghardt et al. assessed human cardiac RLC for sensitivity to mutation with M20L, E134A, and G162R. Measurements using photoactivatable GFP tagged RLC (RLC-PAGFP) exchanged into permeabilized papillary muscle fibers showed that disease-linked mutants invert intermediate state occupation favoring lower free-energy in the active cycle when compared to WT. Lower free-energy intermediate occupancy led to lower energy conversion efficiency in the fiber, demonstrating a myosin functional modification characterized at the single molecule level, and in the context of the crowded muscle fiber, linking the HCM phenotype to lower efficiency for ATP free-energy conversion to mechanical work. M20L and G162R are able to produce maximal isometric force with below normal lever-arm stiffness by radically or modestly reconfiguring sub-state populations while E134A cannot maintain normal maximal force/stiffness, thereby compromising actin binding ¹².

Alvarez-Acosta et al. reported on two novel *MYL2* mutations in a cohort of 124 consecutive HCM index patients: **Ile158Leu (I158L)** causing severe hypertrophy with atrial fibrillation and likely pathogenic but related to a good prognosis, and **Asp166Ala (D166A)**, pathogenic and related to obstructive septal asymmetrical hypertrophy with a good prognosis ⁴. No *in vitro* or *in vivo* studies exist reporting on these two new *MYL2* mutations (Fig. 2).

This is in contrast to a thoroughly studied **Asp166Val (D166V)** mutation that occurs at the last amino acid residue of the human cardiac RLC similar to D166A (Fig. 2). It was identified by Richard et al. where it was mistakenly labeled as D166L ⁹⁵. Transgenic D166V mice hearts exhibited fibrotic lesions, reduced level of *in situ* RLC phosphorylation and skinned papillary muscle fibers from transgenic D166V mouse revealed a large increase in the Ca²⁺ sensitivity of contractile force, decreased maximal ATPase and force, profoundly decreased kinetics of force-generating myosin cross-bridges ⁶². Muthu et al. tested the hypothesis that an *ex vivo* phosphorylation of Tg-D166V cardiac muscle may rescue the detrimental contractile phenotypes observed at the level of single myosin molecules and in Tg-D166V papillary muscle fibers ⁸⁶. They showed that MLCK-induced phosphorylation of Tg-D166V cardiac myofibrils and muscle fibers was able to increase the reduced myofibrillar ATPase and reverse an abnormally increased Ca²⁺ sensitivity of force, but not for the maximal force which decreased upon phosphorylation ⁸⁶. This was followed by subsequent experiments to test whether pseudo-phosphorylation (S15D) of cardiac RLC could be used as a rescue method to alleviate a cardiomyopathy phenotype brought about by a disease-causing mutation in the myosin RLC. The S15D substitution at the phosphorylation site of RLC was inserted into the recombinant human cardiac WT and D166V mutant to mimic constitutively phosphorylated RLC proteins ⁸⁷. They showed an S15D-induced rescue of both the enzymatic and binding properties of D166V-myosin to actin, along with a significant increase in force production capacity in the *in vitro* motility assays for S15D-D166V vs. D166V reconstituted myosin as well as a rescue in D166V-elicited abnormal Ca²⁺ sensitivity of force in porcine papillary muscle strips ⁸⁷. The group subsequently tested the S15D-RLC rescue hypothesis *in vivo* Tg-S15D-D166V mice where the human cardiac S15D-D166V construct was substituted for mouse cardiac RLC and

compared to WT and D166V mice in functional, structural, and morphological assessments¹³². Echocardiography and invasive hemodynamic studies demonstrated significant improvements of intact heart function in S15D-D166V mice compared with D166V, with the systolic and diastolic indices reaching those monitored in WT mice. A largely reduced maximal tension and abnormally high myofilament Ca^{2+} sensitivity observed in D166V-mutated hearts were reversed in S15D-D166V mice. Low-angle X-ray diffraction study revealed that altered myofilament structures present in HCM-D166V mice were mitigated in S15D-D166V rescue mice¹³².

Population studies show most HCM-associated RLC mutations to cause hypertrophied left ventricles and diastolic abnormalities. Biophysical and biochemical characterizations of mutated RLC indicate that depending upon the location and the nature of the mutation, stochastic changes in RLC sequence may render changes in the structure, as well as affect enzymatic, binding, and force production capability of myosin. Despite the progress in the identification of RLC pathogenic variants, genotype-phenotype correlations for HCM are incompletely defined which limits the use of genetic test results to guide clinical management.

Myosin ELC (*MYL3*)

The human heart contains two ELC isoforms: the atrial isoform (ELCa), encoded by the *MYL4* gene located on chromosome 17q21-qter, and the ventricular isoform (ELCv), encoded by the *MYL3* gene localized on chromosome 3p21.3-p21.2 which also encodes for the slow-twitch skeletal ELC²⁷. Striated muscle ELC belongs to the EF-hand family of Ca^{2+} binding proteins, but evolutionarily it lost the ability to bind Ca^{2+} ³⁴. The ELC protein is an integral structural component of the actomyosin cross-bridge; however, little is known about the functional significance of the cardiac-specific isoform, containing a unique ~43 amino acids / 91Å long N-terminus (N-ELC)¹⁰. The N-ELC is comprised of a lysine-rich actin binding region that was shown by many to make direct molecular contacts with actin during actin-myosin interaction and muscle contraction^{40, 57, 81, 82, 108, 117-119, 128, 130}. The N-ELC was also proposed to interact with actin by binding to the SH3 domain of myosin heavy chain during the ATPase cycle^{67, 70} and to modulate the weak-to-strong structural transition³⁸. Regardless of whether the effect of the ELC/actin interaction on the actomyosin ATPase cycle proceeds through the SH3 domain or through a direct N-ELC-actin binding, the finding that the N-ELC may bind to SH3 suggests that the conformation, and thus the function of N-ELC may be highly sensitive to any structural modifications (e.g. due to mutation) in the entire myosin molecule. It was proposed to function as a tether between the myosin head and actin capable of regulating the thick and thin filament interactions and force production in the heart⁵⁷. It was also proposed to use a novel mechanism of step frequency modulation to control myosin power output and cardiac myosin power generation *in vivo*^{13, 127, 128}. The C-terminal region of ELC interacts with the MHC and possibly with the RLC⁴⁶. The C-terminus of ELC contains Ser195, a potential phosphorylation site on the cardiac ELC^{9, 50}, but the role of ELC phosphorylation in heart function is totally unknown¹¹³. Studies in zebrafish model suggested that ELC phosphorylation may have a regulatory role in the heart as the inability of ELC to be phosphorylated at Ser195 in the *laz*^{+/-} mutant led to contractile defects and cardiac death¹⁰¹.

MYL3 mutations

Similar to other sarcomeric proteins, mutations in the ventricular ELC (encoded by *MYL3*) are implicated in cardiomyopathy diseases. Compared to β -MHC or myosin binding protein-C (MyBP-C), mutations in the ELC are quite rare, but they are also associated with malignant outcomes^{56, 64, 83, 89, 92, 95}, and they represent a largely understudied area of research. To date, 13 mutations in *MYL3* have been identified by population studies to cause HCM. Among them, 5 missense mutations are located in exon 3 (E56G, A57G, R63C, V79I, R81H), 6 in exon 4 (G128C, E143K- HCM with restrictive physiology, M149V, E152K, R154H, H155D) and 2 in exon 5 (M173V, E177G) (Fig. 3). Interestingly, all ELC mutations are located in the non-functional EF-hand Ca^{2+} binding motifs of the ELC molecule with the majority located in the C-terminus ELC (Fig. 3). The mutations have been shown to cause various HCM phenotypes in humans, from asymmetric septal hypertrophy (A57G, V79I, R63C), MVC obstruction (M149V, R154H) to non-symptomatic course of the disease (in cases of patients heterozygous for E143K). They have been mostly associated with adult-onset HCM, but several are also observed as infantile/childhood forms (R154H, E177G, E143K and M173V). Some ELC mutations are also associated with SCD at a young age (A57G, E143K, and M149V).

Glu56Gly (E56G) was identified together with other HCM-linked casual mutations in the nine genes encoding for sarcomeric proteins including ELC⁹⁵. The *MYL3* gene accounted for < 0.5% of all analyzed cases with only one E56G mutation in ELC⁹⁵. Six families presented with more than one mutation (double heterozygous, compound heterozygous, and homozygous mutations), and phenotypic analyses suggested a gene dose effect in these patients⁹⁵. The E56G mutation is located in exon 3 of ELC (Fig. 3). A comparison of various ELC sequences demonstrated that the glutamic acid in position 56 is highly conserved across species and tissues⁴². Biophysical studies using time-resolved FRET measurements by Thomas group showed that the E56G mutation may alter the distribution of structural states of the actin-myosin complex, perturbing the weak to strong cross-bridge transition, which is a primary component of force generation³⁹. To gain a better understanding on pathological changes leading to the development of HCM by E56G, Lossie and colleagues generated two mouse models with cardiomyocyte-specific overexpression of the non- (TgM^{hVLC-1}) and E56G- mutated (TgM^{E56G}) human ventricular ELC⁶⁹. Echocardiography and gene expression assessment of hearts of 3 month-old TgM^{E56G} showed no signs of cardiac hypertrophy and no changes in hypertrophic markers. Maximal left ventricular pressure development of isolated perfused hearts *in vitro* prepared from TgM^{E56G} was significantly lower compared with TgM^{hVLC-1}. Differences between mutant and normal mice were also observed at the sarcomere level - in experiments on skinned fibers and myosin. Laser trap and *in vitro* motility assays demonstrated reduced stiffness, force generation and actin sliding velocity of myosin molecules prepared from E56G mice compared with control myosin. Maximal isometric force obtained at the pCa 4.5 of skinned fibers was significantly lower for TgM^{E56G} mice compared with control mice while Ca^{2+} sensitivity was not affected. Observed deleterious effects of the E56G mutated ELC on the myosin function suggested that E56G mutation may cause HCM by means of weakened myosin-lever arm affinity, reduced stiffness and force generation, slowed actin

filament sliding velocity and the depressed cardiac performance. The resulting cardiac hypocontractility could be activating hypertrophic pathways responsible for the development of E56G-induced HCM phenotype ⁶⁹.

Ala57Gly (A57G) was discovered in two unrelated Korean families and one Japanese man diagnosed with HCM ⁶⁴. The mutation is located in exon 3 of ELC (Fig. 3) and similar to the E56 residue, it is highly conserved in other ELC isoforms across species ⁴². The phenotype associated with this mutation entailed classic asymmetric septal hypertrophy and SCD, with pathology and disease progression varying among siblings and other family members ⁶⁴. A study by Lossie et al. using protein-binding experiments by surface plasmon resonance showed the A57G-mediated disturbed affinities to myosin heavy chain (significantly lower binding to head-rod fragment reflected by 2-fold higher K_D) ⁶⁸. The A57G mutation was associated with higher myofilament Ca^{2+} -sensitivity in mice causing pathological cardiac remodeling and increased cardiac output and stroke work ⁵⁹. Using particle tracking of Qdot-labeled actin in the standard *in vitro* motility assay ¹²⁷, and measuring force-velocity for single and ensemble myosins ^{14, 15, 127}, it was shown that compared to WT, the A57G and E143K ELC mutations had high and rising duty ratio with increasing load implying that the fraction of force producing myosin is higher and growing during contraction phase ¹²⁸. Interestingly, force-velocity measurements demonstrated an upregulation of power for A57G myosin and downregulation for E143K ¹²⁸. Ensemble motility and single myosin mechanical characteristics were shown to be consistent with A57G that impairs the N-ELC - actin binding ¹²⁸. In transgenic animals, A57G mutation led to pathological cardiac morphology, including extensive disorganization of myocytes and substantial interstitial fibrosis, most likely due to accumulation of collagen aggregates, as well as led to a decrease in lattice spacing in small angle X-ray diffraction study in Tg-A57G papillary muscle fibers but had no effect on the crossbridge mass distribution ⁸⁵. The structural alterations were paralleled by mechanical changes resulting in increased stiffness/passive tension, increased Ca^{2+} sensitivity of force and decreased maximal tension in skinned papillary muscle from Tg-A57G mice ⁵⁹. In addition, Tg-A57G animals showed a significant upregulation of hypertrophic markers (ANP, BNP, collagen VIIIa) that were activated upon exercise, along with a visible increase in heart size and occurrences of fibrosis, especially in the interventricular septum ⁶⁰. Proteomic analysis of Tg-A57G hearts demonstrated alterations in Ca^{2+} homeostasis along with fatty acid metabolism, suggesting these may be important in the pathological cardiac remodeling observed in Tg-A57G myocardium ³³.

Arg63Cys (R63C) mutation was identified during a systematic mutation screening of 38 Taiwanese patients diagnosed with HCM ¹⁷. It was found in a 32-year-old proband with the phenotype of asymmetric septal hypertrophy with LV outflow tract pressure gradient of 52 mmHg (obstructive HCM), intraventricular conduction defects and dyspnea. Additional screening revealed other 2 out of 7 members of the family carrying the mutation and experiencing ECG abnormalities. The R63C mutation is located in the α -helix of the first EF-hand ELC domain (Fig. 3), and the amino acid sequence alignment shows a high degree of conservation of Arg63 across species. No *in vitro* or *in vivo* studies report on this new *MYL3* mutation.

Val79Ile (V79I) mutation was identified in 38-year-old asymptomatic HCM patient referred for clinical evaluation due to a cardiac murmur⁶. Echocardiography analysis showed classic asymmetric ventricular hypertrophy. Additional screening revealed that 9 members of the family were positive for V79I mutation, with three displaying borderline HCM phenotype (ECG and/or echocardiographic abnormalities) but they did not fulfill diagnostic criteria for HCM. V79I is located in the region of contact between the ELC and the myosin heavy chain in the first IQ motif on MHC lever arm (Figs. 3 and 4). Similar to other ELC mutations, Val79 is highly conserved across species and tissues.

Arg81His (R81H) mutation was found during a genetic screening of 122 unrelated patients with HCM (64 with a positive family history and 58 with unremarkable or unknown family history), but the clinical information on the specific disease phenotype is not available²⁸. No research has been performed on this ELC mutation.

Gly128Cys (G128C) mutation was found during a genetic screening of 10 HCM-causing genes of 26 patients transplanted for end-stage HCM³⁰. Out of all the patients, one had a novel missense mutation located in the exon 4 of *MYL3*. However, no further phenotypic information was provided in this study. No research findings are available for this ELC mutation.

Glu143Lys (E143K) mutation was found in a young proband homozygous for the mutation who was undergoing cardiac evaluation administered after the premature death of his two younger siblings⁸⁹. An incidence of early death, at 7 years of age, from unknown causes, was reported in a distant relative⁸⁹. The proband demonstrated a phenotype of LV hypertrophy and ECG abnormalities. An unusual variant of HCM was identified, characterized by LV hypertrophy with mild dynamic obstruction in systole⁸⁹. The medical records for deceased brothers indicated they had cardiomyopathy with dilated atria, suggesting restrictive heart disease. The proband's sister and parents, who were heterozygous for the E143K mutation were asymptomatic and their ECG findings were normal⁸⁹. In contrast, family members homozygous for E143K manifested severe HCM in childhood. In another study, 22-year-old female carrying E143K mutation was diagnosed with RCM and class III/IV heart failure while awaiting heart transplantation¹⁶. Electrocardiogram and transthoracic echocardiogram revealed severe biatrial enlargement with preserved biventricular systolic function and no LV hypertrophy, while the Doppler assessment indicated advanced LV diastolic dysfunction, all features reminiscent of RCM¹⁶. In addition, elevated right- and left-sided filling pressures and myofibrillar disarray with interstitial fibrosis were found. E143K mutation is the only one in the group of HCM ELC mutations showing restrictive heart physiology and therefore classifying it as an RCM mutation. The E143K occurs at an evolutionarily conserved residue across species and in various ELC isoforms⁴². The mutation is found in the exposed loop of the EF3-hand motif of ELC^{46, 94} (Fig. 3). In the studies by Lossie et al, E143K mutant showed significantly weaker binding to the myosin lever arm (2-fold higher K_D)⁶⁸. Studies using transgenic E143K mice showed diastolic dysfunction, histopathological changes, fibrosis and sarcomeric irregularities with Z-lines streaming and M-lines vanishing¹³³. Gene expression profiles showed upregulation of hypertrophic markers (collagen I and III and ANF). Data obtained from skinned papillary muscles showed an increased ability of myosin to generate

maximal force, increased $V_{\max}$ of actin-activated ATPase, suggesting that E143K may lead to hypercontractile myosin motor behavior¹³³. Particle tracking Qdot *in vitro* motility assays showed high duty ratio for E143K but force-velocity measurements demonstrated downregulation of power, opposite to what was observed for A57G ELC mutation¹²⁸. However, single-molecule assessments of myosin power in both A57G and E143K were in accordance with the *in vivo* hemodynamic measurements^{59, 133}. In addition, decreased RLC phosphorylation in myofibrils from E143K-ELC hearts was thought to contribute to the E143K-RCM phenotype¹³³.

Met149Val (M149V) mutation was the first mutation in the myosin ELC shown to cause HCM^{23, 92}. Six of the thirteen family members who were positive for the mutation had a rare phenotype of mid-left ventricular chamber thickening due to papillary muscles and ventricular hypertrophy^{23, 92}. Studies suggested that the stretch-activation response of the papillary muscles in the M149V patients might be impaired^{24, 92}. A similar phenotype associated with the M149V mutation in myosin ELC was also reported by Arad et al.⁸. Apical or mid-ventricular hypertrophic cardiomyopathy and multiple cases of SCD at young ages were found in patients harboring this mutation^{8, 74}. The M149V mutation like E143K is located in exon 4 of ELC (Fig. 3) and is shown to be highly conserved across species and tissues⁴². According to the crystal structure (1WDC) by Houdusse et al.⁴⁷, M149V is found in the β -sheet end of the exposed loop region of the EF3 motif of ELC (Fig. 3). M149V mutant showed significantly weaker binding to the myosin lever arm in the studies by Lossie et al. (2-fold higher K_D)⁶⁸. Several transgenic animal models of M149V mutation were created^{52, 99, 122}. Older animals (1-1.5 years of age) replicated the phenotype present in patients carrying this mutation and showed hypertrophied papillary muscles and adjacent ventricular tissue resulting in profound mid-left ventricular cavity obstruction¹²². Interestingly, stretch-activation of response for the papillary muscles of the 3-5 month-old M149V mutant compared to control was distorted. Therefore, it was suggested that molecular defects caused by M149V mutation affect myosin function by disrupting the stretch-activation response of the cardiac papillary muscles and adjacent ventricular tissue through a change that they produce in the elasticity of the neck region of the myosin molecule¹²². Sanbe et al.⁹⁹ showed mild fibrosis, myocyte disarray and loss of sarcomere organization as well as mechanical abnormalities in skinned papillary muscles including increased Ca^{2+} sensitivity and lower maximal force in M149V mice compared with controls. But their animals did not develop mid-ventricular obstruction or hypertrophy, which was the main phenotypic feature of the M149V mutation in the study by Poetter et al.⁹². Another attempt to generate transgenic M149V model was made using rabbits⁵². However, similar to transgenic mice expressing mouse isoform of ventricular ELC, transgenic M149V rabbits did not develop HCM at the structural or functional levels at either the neonatal, juvenile or adult stages⁵².

Glu152Lys (E152K) and **His155Asp (H155D)** mutations were first identified in 2009 by detailed clinical and genetic analysis of 79 patients diagnosed with HCM⁵⁶. The genetic screening led to the identification of novel missense mutations located in exon 4 of *MYL3*. No clinical information was available for these mutations. Likewise, no research findings are available for these ELC mutations.

Arg154His (R154H) mutation of ELC was identified in parallel to M149V by Poetter et al.⁹². Likewise, this mutation also caused an atypical phenotype of hypertrophic cardiomyopathy²³. A young boy was diagnosed with massive mid-left ventricular chamber thickening obstruction; a phenotype that occurs sporadically in children^{23, 92}. As with the other ELC mutations, the R154H is highly conserved in different ELC isoforms and across species⁴². As shown in the crystal structure of scallop myosin (1WDC)⁴⁷, this mutation is located in the exiting helix of the EF3 motif of ELC (Fig. 3). R154H mutant showed significantly weaker binding to the myosin lever arm in the studies by Lossie et al. (3-fold higher K_D) using protein-binding experiments by surface plasmon resonance⁶⁸.

Met173Val (M173V) mutation was identified in an adult proband who had childhood-onset cardiac hypertrophy, but the clinical information on the specific disease phenotype is very limited⁸³. In the absence of animal models of heart disease for M173V, the studies were performed in ELC mutant-exchanged porcine cardiac muscle preparations⁵⁰. Data for M173V-ELC exchanged muscles (~60 %) were compared with ~50 % WT-ELC exchanged porcine cardiac preparations. The M173V mutation was observed to decrease the actin-activated mutant-exchanged porcine myosin ATPase activity and increased calcium sensitivity of force compared with WT-ELC myosin⁵⁰. Interestingly, the S195D-M173V-ELC phosphomimetic protein was able to partially restore the low level of actin-activated myosin ATPase activity that was observed for M173V-ELC myosin⁵⁰. The authors suggested that this C-terminal M173V-ELC mutation may play a critical role in the interaction of the ELC with the C-terminal lever arm region of the myosin cross-bridge and its interaction with actin during force generation and that the C-terminus of ELC is important for the formation of stable ELC-MHC structures and sarcomere assembly⁹¹. Further studies are needed to interrogate the role of S195D-phosphomimetic ELC in abrogating the ELC mutation-induced phenotypes.

Glu177Gly (E177G) mutation similar to M173V is located in the EF4-hand motif of ELC in exon 5 of the *MYL3* gene. Amino acid sequence alignment shows that like all other ELC mutations this residue is highly conserved across species. The E177G mutation was identified in a 3 month-old infant with severe progressive HCM, associated with a paternally inherited mutation in *MYL3*, and leading to his death at 6-month old⁵³. Pathological features of this patient's phenotype included left ventricular concentric hypertrophy and systolic dysfunction even though the father of the infant was entirely asymptomatic. No research findings are available for this E177G mutation in ELC.

In summary, all discussed ELC mutations are located in the non-functional EF-hand Ca^{2+} binding motifs of the myosin ELC (Fig. 3). The majority is located in the N and C-terminus ELC, known to make contact with actin and MHC respectively. The associated phenotypes vary by mutation and the limited availability of animal models carrying these mutations have made it difficult to further characterize the molecular mechanisms underlying these phenotypes.

MLC variants of unknown significance

A pathogenic mutation bears the following criteria: co-segregation with the HCM phenotype (such as LVH) in family members; previously reported or identified as a cause of HCM; absent from unrelated and ethnic-matched normal controls; protein structure and function is importantly altered (for example, frame shift with truncation); and amino acid sequence change in a region of the protein otherwise highly conserved through evolution (no variation observed) among species ⁷⁷. There are many substitutions in the DNA sequence that do not actually cause disease and are therefore regarded as benign polymorphisms. And hence, despite applying all these pathogenicity criteria, the relevance of such minority of variants for causing disease remains unclear. Such mutations are designated into an ambiguous category, variants of uncertain significance (VUS), and have virtually no clinical utility for family screening. Unlike *MYH7* and *MYBPC3*, HCM linked ELC and RLC mutations are quite rare and therefore not many studies are available. However, there are studies showing rare or novel variants for which disease pathogenicity have not yet been demonstrated (and hence classified as VUS). Ma et al. described a missense VUS in ELC with contradictory findings with the goal of determining the pathogenicity of this variant using a combined CRISPR/Cas9-iPSC approach ⁷². The group screened for any VUS in a dozen of individuals and found one individual with one variant (A57D) annotated as “likely pathogenic” but did not find any abnormalities in gene expression or morphological phenotypes associated with HCM. Other such *MYL3* variants found in HCM phenotypes include splice acceptor variants (such as c.560-2delA), intron variants (c.560-3C>T, c.482-6T>C, c.481+5G>A), missense variants (Glu177Gly, Met173Thr, Arg154Cys, Lys142Glu), and synonymous variant (c.219C>T). Similarly, *MYL2* variants in HCM phenotype with uncertain significance include missense variants (Asn101Ser, Ala127Val, Ala140Thr Ala141Thr, Pro143Leu, Pro143Thr, Glu163Gln), synonymous variants (Gly148=), intron variants (c.402+6G>C, c.353+6T>A) ([ClinVar](#)). Many such variants need to be further studied in order to establish any meaningful disease relevance and clinical utility.

Potential mechanisms of MLCs mutation elicited heart remodeling and failure

The advantage of using transgenic animal models of cardiomyopathy lies in the ability to explore the disease phenotypes across multiple scales of organization, ranging from single molecules, myofilament ensemble, skinned and intact muscle fibers and the whole heart. One has to integrate the molecular defects at the level of sarcomeres with the hemodynamic, contractile and energetic responses of the heart. This complex data integration would not be possible without the use of genetic models of hypertrophic, dilated or restrictive cardiomyopathy that recapitulate clinical phenotypes of heart disease. Transgenic mouse models of *MYL2* (RLC) and *MYL3* (ELC)-related cardiomyopathy provide invaluable insights into molecular mechanisms of MLC mutant-dependent alterations in myosin motor function that can ultimately lead to the development of light chain-specific therapeutic modalities. Two latent mechanisms associated with MLC mutation-induced alterations of myosin motor function in heart disease are related to the role of myosin RLC phosphorylation at Ser15 (Fig. 4A) and the N-ELC (Fig. 4B) in modifying the myosin-actin

interaction and heart contractility. Research reports demonstrate that maintaining the physiological levels of RLC phosphorylation is critical for the normal function of the heart and suggest that the myocardium containing dephosphorylated myosin has a reduced ability to produce force and maintain cardiac function at physiological levels. Phosphorylation of myosin RLC has been proposed to play a potential protective role in cardiomyopathy disease that involves alterations of the SRX state and the phosphorylation-induced shift in the SRX $\leftrightarrow$ DRX equilibrium toward the DRX state in which myosin heads can readily interact with thin filaments and produce force ⁴⁵. The mechanism underlying myosin ELC function builds on the studies from our and other laboratories demonstrating that the cardiac-specific N-ELC may play important roles in regulating myosin motor function and force production in muscle ^{38, 57, 70, 80, 85, 125–127, 133}, including mutant-mediated regulation of the SRX $\leftrightarrow$ DRX equilibrium ^{7, 106}. It would be interesting to test whether mutations in MLC that are associated with HCM ^{59, 62, 132} and RCM ¹³³ shift the equilibrium from a myosin OFF-state (SRX)⁴⁵ to the myosin ON state (DRX) ⁷, explaining their hypercontractile phenotypes while those associated with DCM ¹³⁴ favor the OFF-state explaining their clinical hypo-contractility.

Conclusions

The goal of this review is to summarize up-to-date studies on the pathophysiology of HCM, RCM and DCM linked with RLC/ELC mutations. Due to genetic heterogeneity as well as phenotypic variability in HCM, there is no clear distinction/correlation between the location of the mutation on the proteins and the consequent phenotypes. In general, HCM mutations are known to induce a hypercontractility phenotype ¹⁰⁵ (with exceptions such as R58Q that shows a hypocontractile phenotype with SRX stabilization) and are associated with an increase in Ca²⁺ sensitivity of force ⁹³ and occurrences of fibrosis and myofibrillar disarray ¹¹⁶. Such an increase in Ca²⁺ sensitivity of force is observed in RLC- E22K ⁹⁹, K104E ⁴⁸ and D166V ⁶², and ELC-A57G ⁵⁹ while fibrosis is evident in RLC-A13T ⁵⁸, N47K and R58Q ¹²⁴, K104E ⁴⁸, and D166V ⁶², and ELC-A57G ⁵⁹. It is difficult to summarize consistent findings in DCM and RCM models between ELC/RLC mutations as there is only one known DCM mutation in RLC (D94A) and one known RCM associated mutation in RLC (G57E) and ELC (E143K). It is important to note that for many of the mutations described in this review, there is a profound lack of mechanistic, *in vitro* recombinant or *in vivo* animal models, making it difficult to summarize consistent findings between ELC/RLC mutations. Creating transgenic animal models in the future would help in understanding the unknown mechanisms behind some of these poorly studied mutations. A potential limitation of using transgenic mouse models may arise from the expression of α -MHC exclusively in the ventricles. Hence, there is a greater interest in using larger animal models (e.g. pigs or rabbits) as they express β -MHC predominantly in the ventricles ⁷¹, which may bear more clinical implications for translational therapies.

Future directions

Using different experimental animal models of heart disease, studies can be designed to provide novel insights into complex genotype-phenotype relationships in MLCs-related HCM, DCM, and RCM. Investigations of heart remodeling performed at the level of myosin

molecules, myofilaments, and the organ level may reveal the MLC-and mutant-specific disease mechanisms underlying specific cardiomyopathy phenotypes. Understanding the molecular triggers of MLCs-dependent HCM, DCM and RCM is critical for the identification of potential therapeutic measures. Future studies are needed to test whether myosin RLC and possibly ELC phosphorylation and N-ELC related heart remodeling can serve as potential novel therapeutic targets to battle heart disease.

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Figure 1. Tertiary structure of human beta-cardiac heavy meromyosin interacting-heads motif (PDB: 5TBY) obtained by homology modeling (using Swiss-model) of human sequence from aphonopelma homology model (PDB: 3JBH).
The myosin heavy chain (MHC) is indicated with blue ribbon, myosin regulatory light chain (RLC) with magenta ribbon and myosin essential light chain (ELC) in red ribbon.

Figure 2. Schematic representation of *MYL2* (genomic and protein) of the human cardiac myosin regulatory light chain (RLC).

The non-coding regions of *MYL2* are highlighted in black, and the location of mutations are indicated with stars. EF-hand domains and the MLCK-phosphorylation site are depicted in the amino-acid sequence. The structural domains are denoted with red blocks (α -helix) and blue arrows (β -strand). Modified from Bonne et al. *Circ Res* 83: 580–593, 1998.

Figure 3. Schematic representation of *MYL3* (genomic and protein) of the human cardiac myosin essential light chain (ELC).

The non-coding regions of *MYL2* are highlighted in black, and the location of mutations are indicated with stars. EF-hand domains and the MLCK-phosphorylation site are depicted in the amino-acid sequence. The structural domains are denoted with red blocks (α -helix) and blue arrows (β -strand). Modified from Bonne et al. *Circ Res* 83: 580–593. 1998.

Figure 4. Mutations in myosin *MYL2* (A) and *MYL3* (B) in the background of human beta-cardiac heavy meromyosin interacting-heads motif (PDB: 5TBY). Cardiac MLCK-dependent phosphorylation site at Ser-15 (**S15**) is emphasized in myosin RLC (A) and the **N-ELC** region in myosin ELC (B).

Table 1.

Overview of mutations in myosin light chains associated with cardiomyopathies

Gene/Protein	Phenotype	Mutation	References to population studies
<i>MYL2</i> /KLC	HCM	A13T, F18L, M20L, E22K, N47K, R58Q, D94A, P95A, K104E, E134A, I158L, G162R, D166A, D166V, IVS6-1, IVS5-2,	Poetter et al. ⁹² , Flavigny et. al. ²⁶ , Kabaeva et al. ⁵⁴ , Olivotto et. al. ⁸⁸ , Santos et al. ¹⁰⁰ , Anderson et. al. ⁵ , Richard et al. ⁹⁵ , Álvarez-Acosta et al. ⁴ , Barth et al. ¹¹
	RCM	G57E	Caleshu et al. ¹⁶
	DCM	D94A	Huang et al. ⁴⁹ ,
<i>MYL3</i> /ELC	HCM	E56G, A57G, R63C, V79I, R81H, G128C, M149V, E152K, R154H, H155D, M173V, E177G	Richard et al. ⁹⁵ , Lee W. et al. ⁶⁴ , Chiou et al. ¹⁷ , Andersen et al. ⁶ , Fokstuen et al. ²⁸ , Garcia-Pavia et al. ³⁰ , Poetter et al. ⁹² , Epstein ²³ , Arad et al. ⁸ , Kaski et al. ⁵⁶ , Morita et al. ⁸³ , Jay et al. ⁵³
	RCM	E143K	Olson et al. ⁸⁹ , Caleshu et al. ¹⁶
	DCM	Not reported	