Cardiovascular Research (2008) **77**, 659–666
doi:10.1093/cvr/cvm084

Review

Sarcomeric proteins and inherited cardiomyopathies

Sachio Morimoto*

Laboratory of Clinical Pharmacology, Kyushu University Graduate School of Medicine, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Received 8 October 2007; revised 8 November 2007; accepted 19 November 2007; online publish-ahead-of-print 4 December 2007

Time for primary review: 17 days

KEYWORDS
Sarcomere;
Gene mutation;
Hypertrophic cardiomyopathy;
Dilated cardiomyopathy;
Restricted cardiomyopathy

Over the last two decades, a large number of mutations have been identified in sarcomeric proteins as a cause of hypertrophic, dilated or restrictive cardiomyopathy. Functional analyses of mutant proteins *in vitro* have revealed several important functional changes in sarcomeric proteins that might be primarily involved in the pathogenesis of each cardiomyopathy. Creation of transgenic or knock-in animals expressing mutant proteins in their hearts confirmed that these mutations in genes for sarcomeric proteins induced distinct types of cardiomyopathies and provided useful animal models to explore the molecular pathogenic mechanisms and potential therapeutics of cardiomyopathy *in vivo*. In this review, I discuss the functional consequences of mutations in different sarcomeric proteins found in hypertrophic, dilated, and restrictive cardiomyopathies in conjunction with their effects on cardiac structure and function *in vivo* and their possible molecular and cellular mechanisms, which underlie the pathogenesis of these inherited cardiomyopathies.

1. Introduction

Cardiomyopathy is classified into four main forms, hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), and arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C).[1] HCM and DCM increase myocardial mass with distinct patterns of ventricular remodeling. HCM produces ventricular wall thickening (i.e. hypertrophy), especially in the interventricular septum, with decreases in ventricular chamber volumes. DCM produces a prominent increase in chamber volumes as well as ventricular wall thickening. In HCM, systolic function is increased or at least preserved, while diastolic function is impaired in part because of the hypertrophy itself, interstitial fibrosis, and/or myocyte disarray. Diastolic dysfunction is thought to be responsible for symptoms of heart failure and premature sudden cardiac death of HCM patients. In contrast, DCM is characterized by systolic dysfunction, which often leads to heart failure requiring cardiac transplantation or sudden cardiac death. RCM is characterized by restrictive diastolic dysfunction (i.e. restrictive filling and reduced diastolic volume of either or both ventricles) with normal or near normal systolic function and wall thickness.

Since the discovery of an HCM-causing mutation in the gene for β-myosin heavy chain (β-MyHC) at 1990,[2] >400 mutations that cause HCM, DCM, and RCM have been found in the genes for proteins constituting the sarcomere of cardiac muscle, whereas no sarcomeric protein genes have been discovered to be responsible for ARVD/C (*Figure 1* and *Table 1*). Despite a great number of studies that have been done to elucidate the structure and function of normal sarcomeric proteins since the middle of the last century, it is still difficult to predict the exact functional consequences of mutations found in cardiomyopathies only from their nature and position. Discoveries of cardiomyopathy-causing mutations in sarcomeric proteins have led to extensive studies on their functional consequences *in vitro* using varieties of analyzing techniques refined over more than a half century. Furthermore, transgenic or knock-in animal models have extensively been used to explore the physiological function of mutant sarcomere proteins *in vivo* and its involvement into the pathogenesis of cardiomyopathies. These lines of studies are dramatically improving our views of cardiomyopathies as well as the physiological function of sarcomere proteins.

This review focuses on the mutations in sarcomeric proteins, i.e. the thin and thick filament proteins,[3] titin,[4], and Z-disc proteins,[5] and their roles in the pathogenesis of cardiomyopathies. Mutations in sarcolemmal transmembrane proteins, cytoskeletal proteins and nuclear envelope proteins are the other important causes of DCM and are being discussed elsewhere.[6]

2. Mutations in genes for the thin filament proteins

2.1 Troponin complex

2.1.1 Cardiac troponin T

2.1.1.1 cTnT mutations in HCM

Twenty-seven mutations in the cardiac troponin T (cTnT) gene (*TNNT2*) have been found to cause HCM. HCM patients

* Corresponding author. Tel: +81 92 642 6081; fax: +81 92 642 6084.
E-mail address: morimoto@med.kyushu-u.ac.jp

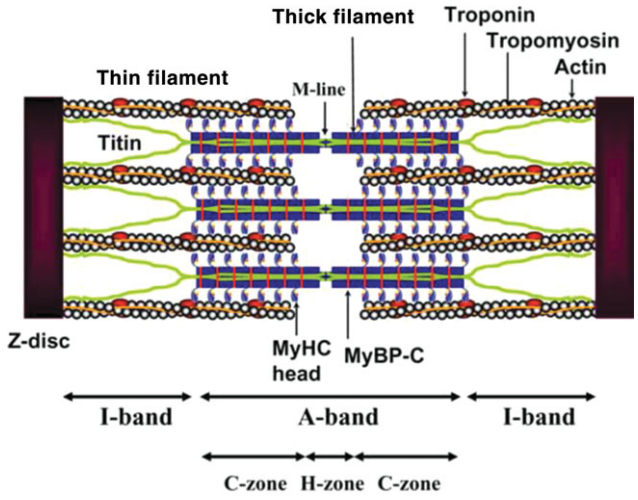

Figure 1 Schematic drawing of sarcomere structure. Tropomyosin forms an α-helical coiled-coil double strand lying along the grooves of actin double strands through head-to-tail polymerization. Troponin complex attaches to a specific region of each tropomyosin and is distributed at regular intervals of 38 nm in the thin filament. Myosin heavy chain (MyHC) has a globular head domain containing actin binding and ATP hydrolytic sites. Myosin-binding protein C (MyBP-C) is a thick filament associated protein, which forms 7–9 transverse stripes at regular intervals of 43 nm in the C-zone of the sarcomere A-band. Titin is a giant protein constituting the third myofilament spanning entire half of the sarcomere from Z-disc to M-line.

Table 1 Numbers of mutations in sarcomeric proteins found in cardiomyopathies

Protein (gene)	HCM	DCM	RCM
Thin filament proteins			
Cardiac troponin T (*TNNT2*)	27[a]	6[a]	–
Cardiac troponin I (*TNNI3*)	26[a]	1[32]	6[a]
Cardiac troponin C (*TNNC1*)	1[a]	1[a]	–
α-Tropomyosin (*TPM1*)	11[a]	2[a]	–
α-Cardiac actin (*ACTC1*)	7[a]	2[a]	–
Thick filament proteins			
β-Myosin heavy chain (*MYH7*)	167[a]	11[a]	–
Ventricular regulatory light chain (*MYL3*)	4[a]	–	–
Ventricular esential light chain (*MYL2*)	10[a]	–	–
Cardiac myosin-bining protein C (*MYBPC3*)	134[a]	–	–
Titin and Z-disc proteins			
Titin (*TTN*)	2[a]	6[a]	–
T-cap (*TCAP*)	4[a,85]	2[a,84]	–
MLP (*CSRP3*)	3[a]	1[a]	–
Myozenin-2 (*MYOZ2*)	1[89]	–	–
α-Actinin (*ACTN2*)	–	1[91]	–
Obscurin (*OBSCN*)	2[90]	–	–
Cypher (*LDB3*)	–	3[a,92]	–

[a]Human Gene Mutagenesis Database at the Institute of Medical Genetics in Cardiff (http://www.hgmd.cf.ac.uk/ac/index.php).

with cTnT mutations show moderate or no significant cardiac hypertrophy in spite of their malignant prognosis, i.e. high incidence of sudden cardiac death.[7] Missense mutations I79N and R92Q, a deletion mutation ΔE160, and a splice donor site mutation intron $16G_1 \rightarrow A$ develop a similar malignant clinical phenotype, with the life expectancy of patients being ∼35 years.[7]

Many studies have been made on the functional aspects of these cTnT mutant proteins, and functional consequences primarily involved in the molecular pathogenic mechanism have been rapidly clarified, although some controversial observations have been reported. Early studies using myocytes transected or infected with mutant cTnT cDNA consistently indicate that the mutations impair muscle contractility and thus may cause compensatory hypertrophy,[8–11] which is not consistent with the current view of the pathogenic mechanism of HCM described follows.

Other types of functional studies indicate that the human cTnTs, when exchanged into the rabbit cardiac skinned muscle fibres or myofibrils, increase the Ca^{2+} sensitivity, without impairing the maximum force-generating capability and ATPase activity.[12,13] Szczesna *et al.*[14] and Harada and Potter[15] reported similar results concerning the mutations I79N, R92Q, R92W, and R92L.

Miller *et al.*[16] created transgenic mice expressing I79N human cTnT and confirmed that the skinned cardiac muscle fibres showed increased myofilament Ca^{2+} sensitivity. Tardiff *et al.*[17] created transgenic mice expressing R92Q human cTnT. Isolated working hearts showed hypercontractility and diastolic dysfunction. Cardiac myocytes showed increased basal sarcomeric activation, impaired relaxation, and shorter sarcomere lengths, indicative of increased Ca^{2+} sensitivity. Chandra *et al.*[18,19] demonstrated that Ca^{2+} sensitivity of myofilaments was enhanced in the mice expressing R92Q, R92W, and R92L cTnTs.

Functional studies so far made strongly suggest that an increased Ca^{2+} sensitivity of cardiac muscle contraction is involved in the pathogenesis of HCM associated with the cTnT mutations.

2.1.1.2 cTnT mutations in DCM

Kamisago *et al.*[20] reported a deletion mutation in *TNNT2*, ΔK210, in two independent families, which is the first cTnT mutation identified to be responsible for familial primary DCM. In a functional study we made, this mutation had a Ca^{2+} desensitizing effect on the force generation in skinned cardiac muscle fibres and the ATPase activity in cardiac myofibrils.[21] Other groups have reported basically the same findings,[22,23] strongly suggesting that the decrease in the Ca^{2+}sensitivity of force generation might be a primary mechanism for the pathogenesis. The second mutation, R141W, that causes DCM was found in a large family.[24] This mutation also decreases the Ca^{2+} sensitivity of force generation without changing maximum force-generating capability, cooperativity and unloaded shortening velocity in skinned cardiac muscle fibres.[25] A quartz-crystal microbalance revealed that this mutation increased the affinity of cTnT for α-tropomyosin (TM).[25] The disease expression of R131W, R205L, and D270N is similar to that of ΔK210 in severity, being characterized by early-onset phenotype, high incidence of sudden death, and/or heart failure, and frequently observed cardiomegaly.[26] Functional studies show that reduced Ca^{2+} sensitivity is a consistent property of these mutations.[27]

We created knock-in mice in which three base-pairs coding for the residue K210 were deleted from endogenous *TNNT2* genes using gene-targeting technology. The knock-in mice developed enlarged hearts and heart failure and showed a high incidence of premature sudden death,[28] closely recapitulating the clinical phenotypes of human patients.[20,26] Skinned cardiac muscle fibres showed a decrease in Ca^{2+} sensitivity of force generation, consistent

with the previous *in vitro* study.[21] Oral administration of pimobendan, a Ca^{2+} sensitizer, known to directly increase the Ca^{2+} sensitivity of myofilaments, was found to reduce the heart size of mutant mice and markedly improve the life expectancy, strongly suggesting that Ca^{2+} sensitizers, such as pimobendan, are beneficial for the treatment of DCM patients affected by this mutation.

2.1.2 Cardiac troponin I

2.1.2.1 cTnI mutations in HCM

Kimura *et al*.[29] reported six mutations in the cardiac troponin I (cTnI) gene (*TNNI3*) that were associated with HCM. Although the prevalence of cTnI mutations is less than that of cTnT mutations, currently 26 mutations have been identified (*Table 1*). Ca^{2+}sensitizing effect on cardiac muscle contraction has commonly been observed for most mutations.[30] Transgenic mice expressing R145G showed the pathological features of typical HCM, including myocyte disarray and interstitial fibrosis, with no significant cardiac hypertrophy.[31] Working heart preparations showed enhanced systolic function and impaired diastolic function. Skinned cardiac muscle fibres showed a significant increase in Ca^{2+} sensitivity with maximum force depression. This mouse model showed a very early death at 13–17 days after birth in mice with mutant cTnI being incorporated into myofilament by ~50%.

2.1.2.2 cTnI mutation in DCM

An N-terminal mutation in cTnI, A2V, has been found to cause a rare case of DCM inherited in an autosomal 'recessive' manner and this mutation impairs the interaction of cTnI with cTnT but not cardiac troponin C (cTnC) as demonstrated in a mammalian two-hybrid assay system.[32]

2.1.2.3 cTnI mutations in RCM

Six missense mutations have been found in the patients with idiopathic RCM.[33] A mixed appearance of RCM and HCM in a family with D190G suggested that there might be a common molecular mechanism for the pathogeneses of RCM and HCM. Functional studies indicate that the six mutations increase the Ca^{2+} sensitivity of force generation in skinned cardiac muscle fibres, with their effects being much greater than those of HCM-causing mutations in cTnI.[34,35] NMR studies show that dramatic Ca^{2+} sensitizing effects are caused by an unexpectedly subtle structural perturbation in a small region within cTnI molecule.[35]

2.1.3 Cardiac TnC

2.1.3.1 cTnC mutation in HCM

A missense mutation L29Q in the cTnC gene (*TNNC1*) was reported in an HCM patient with late onset of the disease.[36] An N-terminal small region containing the L29 residue interacts with an N-terminal small region containing Ser22/23 of cTnI, and this interaction is abolished upon phosphorylation of cTnI Ser22/23 by protein kinase A (PKA), leading to a decrease in the myofilament Ca^{2+} sensitivity.[37] Schmidtmann *et al*.[38] found that this mutation abolished the N-terminal interaction between cTnI and cTnC, leading to a loss of change in myofilament Ca^{2+} sensitivity upon phosphorylation of cTnI by PKA.

2.1.3.2 cTnC mutation in DCM

A missense mutation, G159D, in *TNNC1* was found in a DCM family with a malignant phenotype.[26] Mirza *et al*.[27] reported that this mutation reduced the Ca^{2+} sensitivity in actomyosin ATPase and *in vitro* motility assays. However, this mutation had no effects on the Ca^{2+} sensitivity of force generation when mutant cTnC was exchanged into skinned fast skeletal or cardiac muscle fibres.[39] Alternatively, this mutation has been shown to blunt the Ca^{2+} desensitization of force generation induced by phosphorylation of cTnT Thr203 by PKC or cTnI Ser22/23 by PKA.[40,41]

2.2. Tropomyosin

Striated muscle expresses two isoforms of TM, αTM and βTM, encoded in different genes. Skeletal muscle expresses both isoforms, but cardiac muscle expresses mostly αTM isoform.

2.2.1 αTM mutations in HCM

A total of 11 missense mutations have been found in the αTM gene (*TPM1*) as a cause of HCM (*Table 1*). *TPM1* is an uncommon cause of familial HCM (~5%), except for the Finnish population (~25%).[42] A Ca^{2+} sensitizing effect on skinned cardiac muscle contraction has commonly been observed for most mutations in αTM, as are the cases with cTnT and cTnI mutations.[43–45]

D175N has been found in three families with a favourable prognosis of near normal life expectancy as well as in a family with a malignant prognosis associated with frequent sudden deaths.[46,47] Biopsy samples from slow skeletal muscles of two patients showed an increased Ca^{2+} sensitivity of force generation.[48] Transgenic mice expressing D175N mouse αTM showed a normal life expectancy.[49] Skinned cardiac muscle fibres had increased Ca^{2+} sensitivity, which could account for the diastolic dysfunction in working heart preparations.

Mice expressing E180G 'mouse' αTM showed a very severe and lethal phenotype with no mice surviving beyond 6 months of age.[50] Working heart preparations showed significant diastolic dysfunction and skinned cardiac muscle fibres had increased Ca^{2+} sensitivity. However, transgenic mice expressing E180G 'human' αTM showed a very mild phenotype with apparent normal longevity similar to the clinical phenotype of human patients.[51,52] Nevertheless, skinned single cardiac myocytes showed increased Ca^{2+} sensitivity, which manifested as a significant diastolic dysfunction *in vivo*.

2.2.2 αTM mutations in DCM

Two missense mutations, E40K and E54K, have been identified in the *TPM1* gene as a cause of DCM with a relatively malignant phenotype.[53] Both mutations change the acidic residue into a basic residue. Interestingly, no missense mutations that cause an electrical charge reversal has not been reported in any HCM mutations in αTM. Both mutations decreased the Ca^{2+} sensitivity as demonstrated in studies employing reconstituted actomyosin or myofibrillar ATPase activity and the *in vitro* motility assay.[27,43]

Transgenic mice expressing E54K mouse αTM showed variable phenotype depending on the copy number of transgene.[54] The mice with high-copy number all died within 1.5 months of age. In contrast, mice with moderate-copy number showed a relatively mild phenotype with a tendency

of developing DCM after 2 months of age and starting dying by 4–6 months of age. In skinned cardiac muscle preparations, both moderate- and high-copy transgenic mice demonstrated significant decreases in the Ca^{2+} sensitivity, consistent with the *in vitro* studies, as well as a marked depression in maximum force/cross-sectional area.

2.3. Actin

Mammalian cells express six isoforms encoded in different gene which can be classified into three main groups: α-, β-, and γ-actins. Sarcomeric actins, α-cardiac and α-skeletal, are known to be co-expressed in myocardium.[55,56]

2.3.1 α-Cardiac actin mutations in HCM

The α-cardiac actin gene (*ACTC1*) is a rare cause of HCM (1.5%) and seven missense mutations have been found (*Table 1*). These mutations decreased the thermal stability of actin monomer and impaired the filament formation, suggesting that the inability to form myofilaments and/or the accumulation of aggregates could be one of the cellular pathological effects of *ACTC1* mutations.[57–59] E99K reduced the sliding velocity and averaged force in an *in vitro* motility assay and decreased the affinity of actin for myosin,[59] suggesting that impaired actomyosin interaction is the primary defect at molecular level leading to HCM associated with this mutation.

2.3.2 α-Cardiac actin mutations in DCM

Two missense mutations, R312H and E361G, have been identified in *ACTC1* as a cause of DCM with apparently favourable prognosis.[60] R312H occurs at the residue next to D311 in subdomain 3 that forms an attractive electrostatic interaction with TM in the high Ca^{2+} state.[61] E361G occurs in a binding domain for α-actinin in subdomain 1, which anchors thin filaments to Z-discs and intercalated discs.[62] Wong *et al.*[57] reported that this mutation had no effects on actin polymerization, rigor binding, actomyosin ATPase, and *in vitro* motility, but slightly reduced the affinity of actin filament for α-actinin, suggesting that impaired force transmission via Z-discs and intercalated discs might be responsible for the pathogenesis. Vang *et al.*[58] reported that these two mutations impaired the protein-folding pathway and filament formation, as were the cases with HCM-causing mutations.

3. Mutations in genes for the thick filament proteins

3.1. Myosin

Cardiac muscle expresses two isoforms, α- and β-cardiac MyHCs. The α-MyHC is abundant in both atria and ventricles during mammalian embryogenesis. In small mammals, including mouse and rat, α-MyHC remains a predominant isoform expressed in both atria and ventricles during adulthood.[63] In contrast, β-MyHC is expressed predominantly in the ventricles and α-MyHC in the atria during adulthood in large mammals, including rabbit and human. The β-cardiac MyHC is also known to be expressed in slow skeletal muscle, type I fibres.

3.1.1 β-MyHC mutations in HCM

Mutations in the β-MyHC gene (*MYH7*) are the most frequent causes of HCM, with at least 167 mutations identified in both exon and intron, the majority of which are missense mutations (*Table 1*). R403Q and R453C are associated with a malignant phenotype characterized by early onset, a 100% disease penetrance in adults, and a high incidence of premature sudden death.[64,65]

Geisterfer-Lowrance *et al.*[66] created a knock-in mouse model in which R403Q was introduced into the endogenous α-MyHC gene (*MYH6*). Heterozygous mice were viable, reproduced normally, and lacked overt symptoms. Hearts of young mice showed delayed left ventricular pressure relaxation and chamber filling without gross morphologic or histologic abnormalities, demonstrating that diastolic dysfunction is the primary response to the mutation.[66,67] Gao *et al.*[68] studied calcium cycling in intact cardiac muscle fibres and reported that mutant myofilaments were more sensitive to Ca^{2+} below half-maximal $[Ca^{2+}]_i$ and lead to a diastolic dysfunction. Warshaw and colleagues[69,70] investigated cardiac myosin isolated from homozygous mice and reported that R403Q enhanced the force-generating capacity and actomyosin ATPase activity as well as the velocity of actin filament sliding, whereas R453C enhanced only the force-generating capacity. Although the reason for the discrepancy from the previous studies remains unclear,[71] they proposed that the HCM-causing mutations should augment the power output of the hearts beyond the mechanical stress tolerance of a normal cardiac sarcomere, which might be a primary stimulus for the hypertrophic response.[69]

Marian *et al.*[72] created a transgenic rabbit model expressing human β-MyHC R403Q mutant. These rabbits exhibited the phenotype virtually identical to that of human HCM, i.e. premature death, cardiac hypertrophy, myocyte disarray, interstitial fibrosis, and normal systolic function, so that this model promises to be an important resource for pathogenic and therapeutic studies of human HCM.

3.1.3 β-MyHC mutations in DCM

A total of 11 missense mutations have been found in *MYH7* as a cause of DCM (*Table 1*). Kamisago *et al.*[20] reported two mutations S532P and F746L in families with early-onset phenotype. Schmitt *et al.*[73] created knock-in mouse models with S532P and F746L being engineered into endogenous genes. Heterozygous and homozygous mice were fully viable and fertile, and they survived >1 years. Systolic function was impaired in homozygous but not in heterozygous mice. Cardiac myocytes isolated from heterozygous mice showed impaired contractility. They investigated the cardiac myosin isolated from homozygous mice and reported that S532P reduced the force-generating capacity and the velocity of actin filament sliding, whereas F764L reduced only the actin-activated ATPase activity.[70,73] Based on these findings, they proposed that the compromised enzymatic and/or mechanical activities in cardiac myosin may trigger the cascade of events that lead to DCM.

3.1.4 Essential myosin light chain mutations in HCM

Mutations in the ventricular essential myosin light chain (ELC) gene (*MYL3*) is a rare cause of HCM (<1%), with only four missense mutations being identified (*Table 1*). M149V was found in a large family, of which six had a rare

phenotype, as a familial condition, involving mid-left ventricular chamber thickening.[74] Low mortality rate in this family suggests M149V is associated with a benign prognosis. Transgenic mice expressing human ventricular ELC with M149V faithfully recapitulated the cardiac disease of the patients with this mutation at old age (>1 year) (i.e. an unusual phenotype of mid-cavitary obstruction).[75] Transgenic mice expressing 'mouse' ventricular ELC with M158V, corresponding to M149V in human, developed no hypertrophy, even in senescent animals (1.5 years).[76] Transgenic rabbits expressing rabbit ventricular ELC with M154V, corresponding to M149V in human, again showed no discernible pattern of disease at the structural or functional levels except for a very subtle increase in the myofilament Ca^{2+} sensitivity, suggesting that this mutation is not causative for HCM at least in rabbit, although it remains possible that a phenotype might present in older rabbits.[77]

3.1.5 Regulatory myosin light chain mutations in HCM

Eight missense mutations and two splice site mutations have been found in the ventricular regulatory myosin light chain (RLC) gene (*MYL2*) as a cause of HCM (*Table 1*). The *MYL2* gene has been shown to be common in HCM families with a malignant prognosis.[78]

Szczesna-Cordary *et al.*[79] created transgenic mice expressing E22K, N47K, and R58Q human ventricular RLC. These mice showed no cardiac hypertrophy. Nevertheless, cardiac myofibrils or skinned fibres prepared from E22K and N47K, but not from R58Q mice showed increased Ca^{2+} sensitivity. Dumka *et al.*[80] studied mechanical properties of myosin crossbridges during single-turnover contraction in cardiac myofibrils from mutant mice and reported that E22K had no effect on the mechanical properties of crossbridges.

3.2. Myosin-binding protein C

Mutations in the cardiac myosin-binding protein C (cMyBP-C) gene (*MYBPC3*) are one of the most frequent genetic causes of HCM, with at least 134 different mutations identified in both exon and intron of the gene (*Table 1*). Missense mutations constitute only about half of the mutations and the remaining half include insertions, deletions, and splice donor/acceptor site mutations that are predicted to cause a C-terminal truncation of cMyBP-C molecule. *MYBPC3* mutations is associated with later onset, less hypertrophy, lower penetrance, and a better prognosis compared with mutations in *MYH7* or *TNNT2*.[81]

Transgenic mice expressing a mutant cMyBP-C lacking its C-terminal half, which mimicked the truncation mutations in HCM, showed no significant cardiac hypertrophy and no significantly increased morbidity or mortality.[82] The truncated protein was not correctly incorporated into the A-band of the sarcomere, suggesting that haplo-insufficiency might play a role in the pathogenic process. Homozygous knockout mice were also viable and displayed well-developed sarcomeres, but exhibited significant cardiac hypertrophy with reduced diastolic function. These data demonstrated that cMyBP-C is not essential for forming and maintaining sarcomere ultrastructure, but that the absence of cMyBP-C results in cardiac hypertrophy and dysfunction.[83]

4. Mutations in genes for titin and Z-disc proteins

4.1. Titin

In the titin gene (*TTN*), two missense mutations have been identified as a cause of HCM, and five missense mutations and one frameshift mutation have been identified as a cause of DCM (*Table 1*). Titin has multiple important roles in muscle physiology as a determinant of passive muscle stiffness and myofilament Ca^{2+} sensitivity as well as a biomechanical sensor controlling gene expression.[4] Titin is encoded by a single gene *TTN* and differential splicing of exons encoding the central I-band region produces muscle type-specific isoforms.

The affinity of titin Z1-Z2 domains for T-cap (or telethonin) is decreased by DCM-causing mutation V154M.[84] The affinity of the titin Z-repeat region for α-actinin is increased by HCM-causing R740L while decreased by DCM-causing A743V,[84,85] suggesting that opposite effects on the integrity of titin in the Z-disc might explain the distinct phenotypes caused by these mutations. DCM-causing S3799Y increases the affinity of the titin N2B region for four and half LIM protein 2,[86] known to bind metabolic enzymes,[87] suggesting that altered recruitment of metabolic enzymes to the sarcomere may play a role in the pathogenesis of cardiomyopathies.

4.2. Z-disc proteins

T-cap binds to the N-terminus of titin at the Z-disc. T-cap interaction with titin is stabilized by another Z-disc protein, muscle LIM protein (MLP), and MLP/T-cap/titin complex are thought to serve as a mechanical stress sensor.[88] Four missense mutations have been identified in the T-cap gene (*TCAP*) as a cause of HCM.[89,90] Yeast two-hybrid (Y2H) assays show that HCM-causing mutations, T137I and R153H, enhance the interaction of T-cap with titin as well as myozenin-2 (or calsarcin-1), which tethers calcineurin to the Z-disc.[90] R87Q and E132Q have been identified in two patients with sporadic DCM and Y2H assays show that both mutations impair the complex formation of T-cap with MLP, while E132Q also impairs the interaction with titin and myozenin-2,[88,90] strongly suggesting that altered interactions among the Z-disc mechanical sensor complex are responsible for the disease.

Several missense or frameshift mutations have been found in the MLP gene (*CSRP3*) as a cause of HCM. Y2H assays show that C58G leads to a decreased binding activity of MLP to α-actinin.[91] Only one missense mutation, K69R, in *CSRP3* has been identified as a cause of familial DCM.[92] This mutation is in a nuclear localization signal adjacent to the LIM1 domain of MLP.

A missense mutation, S48P, in the myozenin-2 gene (*MYOZ2*) has been found in a large family with HCM characterized by early onset of symptoms, pronounced cardiac hypertrophy, and cardiac arrhythmias.[93]

A missense mutation, Q9R, in the α-actinin gene (*ACTN2*) has been found in a patient with DCM and reported to disturb the interaction of α-actinin with MLP.[92]

Two missense mutations, R4344Q and A4448T, in the obscurin gene (*OBSCN*) have been found in a patient with HCM and reported to decrease the affinity of obscurin for the titin Z-disc domain.[94]

Three missense mutations, D117N, K136M, and D626N, in the cypher gene (*LDB3*) have been found in patients with DCM.[95,96] Y2H assays and pull-down assays show that D626 N decreases the affinity of the C-terminal LIM domain of cypher for PKC, suggesting that an abnormality in the anchoring-protein of PKC in the Z-disc may play a role in the pathogenesis of DCM.

5. Conclusion

Functional studies that have so far been made on sarcomeric regulatory proteins shows that HCM- or RCM-causing mutations increase the Ca^{2+} sensitivity of cardiac myofilaments, whereas DCM-causing mutations decrease it. Increased Ca^{2+} sensitivity of cardiac myofilaments is also caused by HCM-causing mutations in the thick filament proteins; this is not surprising because it is well known that there exists a positive feedback mechanism between troponin Ca^{2+} binding and myosin crossbridge attachment. Increased myofilament Ca^{2+} sensitivity is expected to increase the ATP utilization by actomyosin at submaximal Ca^{2+} concentrations, which might cause an imbalance in energy supply and demand in the heart under severe stress. NMR studies have revealed that myocardial energetics is compromised in mouse models of HCM caused by R92Q mutation in cTnT and R403Q mutation in α-MyHC,[97,98] as is the case with human patients affected by mutations in sarcomeric proteins, including cTnT.[99] Chandra *et al.*[19] reported that the skinned cardiac muscle fibres from transgenic mice with HCM-causing ΔE160 mutation in cTnT showed an increased ATP consumption of force maintenance (i.e. increased tension cost). Based on the similarity of the clinical phenotypes of diseases that limit myocardial energy production to those of HCM, Ashrafian *et al.*[100] have proposed that the increased energy demand owing to inefficient sarcomeric ATP utilization in HCM compromises the contraction and homeostatic functions of the cardiac myocyte, leading to myocyte hypertrophy.

The increase and decrease in the myofilament Ca^{2+} sensitivity well account for the diastolic and systolic dysfunction of model animals as well as human patients of HCM and DCM, respectively. Diastolic and systolic dysfunction should increase and decrease the ventricular wall stress, which could be transmitted to the biomechanical sensor in Z-discs or intercalated discs controlling gene expression and lead to HCM (or RCM) and DCM, respectively. Further studies on the detailed pathogenic mechanisms involving the huge numbers of mutations of sarcomeric proteins found in cardiomyopathies will contribute to clarifying the entire feature of the physiological mechanisms controlling cardiac function and structure.

Conflict of Interest: none declared.

Funding

This work was supported by a grant from the Vehicle Racing Commemorative Foundation.

References

1. Richardson P, McKenna W, Bristow M, Maisch B, Mautner B, Oconnell J *et al*. Report of the 1995 World Health Organization International Society and Federation of Cardiology Task Force on the definition and classification of cardiomyopathies. *Circulation* 1996;**93**:841–842.
2. Geisterferlowrance AAT, Kass S, Tanigawa G, Vosberg HP, McKenna W, Seidman CE *et al*. A molecular-basis for familial hypertrophic cardiomyopathy - a β-cardiac myosin heavy-chain gene missense mutation. *Cell* 1990;**62**:999–1006.
3. Gordon AM, Homsher E, Regnier M. Regulation of contraction in striated muscle. *Physiol Rev* 2000;**80**:853–924.
4. Granzier H, Wu Y, Siegfried L, LeWinter M. Titin: physiological function and role in cardiomyopathy and failure. *Heart Fail Rev* 2005;**10**: 211–223.
5. Frank D, Kuhn C, Katus HA, Frey N. The sarcomeric Z-disc: a nodal point in signalling and disease. *J Mol Med* 2006;**84**:446–468.
6. Fatkin D, Graham RM. Molecular mechanisms of inherited cardiomyopathies. *Physiol Rev* 2002;**82**:945–980.
7. Watkins H, McKenna WJ, Thierfelder L, Suk HJ, Anan R, O'Donoghue A *et al*. Mutations in the genes for cardiac troponin T and alpha-tropomyosin in hypertrophic cardiomyopathy. *N Engl J Med* 1995;**332**: 1058–1064.
8. Sweeney HL, Feng HS, Yang Z, Watkins H. Functional analyses of troponin T mutations that cause hypertrophic cardiomyopathy: insights into disease pathogenesis and troponin function. *Proc Natl Acad Sci USA* 1998;**95**:14406–14410.
9. Watkins H, Seidman CE, Seidman JG, Feng HS, Sweeney HL. Expression and functional assessment of a truncated cardiac troponin T that causes hypertrophic cardiomyopathy. Evidence for a dominant negative action. *J Clin Invest* 1996;**98**:2456–2461.
10. Marian AJ, Zhao G, Seta Y, Roberts R, Yu Q-t. Expression of a mutant (Arg92Gln) human cardiac troponin T, known to cause hypertrophic cardiomyopathy, impairs adult cardiac myocyte contractility. *Circ Res* 1997; **81**:76–85.
11. Rust EM, Albayya FP, Metzger JM. Identification of a contractile deficit in adult cardiac myocytes expressing hypertrophic cardiomyopathy-associated mutant troponin T proteins. *J Clin Invest* 1999;**103**: 1459–1467.
12. Morimoto S, Yanaga F, Minakami R, Ohtsuki I. Ca^{2+}-sensitizing effects of the mutations at Ile-79 and Arg-92 of troponin T in hypertrophic cardiomyopathy. *Am J Physiol* 1998;**275**:C200–C207.
13. Yanaga F, Morimoto S, Ohtsuki I. Ca^{2+} sensitization and potentiation of the maximum level of myofibrillar ATPase activity caused by mutations of troponin T found in familial hypertrophic cardiomyopathy. *J Biol Chem* 1999;**274**:8806–8812.
14. Szczesna D, Zhang R, Zhao J, Jones M, Guzman G, Potter JD. Altered regulation of cardiac muscle contraction by troponin T mutations that cause familial hypertrophic cardiomyopathy. *J Biol Chem* 2000;**275**: 624–630.
15. Harada K, Potter JD. Familial hypertrophic cardiomyopathy mutations from different functional regions of troponin T result in different effects on the pH and Ca^{2+} sensitivity of cardiac muscle contraction. *J Biol Chem* 2004;**279**:14488–14495.
16. Miller T, Szczesna D, Housmans PR, Zhao J, de Freitas F, Gomes AV *et al*. Abnormal contractile function in transgenic mice expressing a familial hypertrophic cardiomyopathy-linked troponin T (I79N) mutation. *J Biol Chem* 2001;**276**:3743–3755.
17. Tardiff JC, Hewett TE, Palmer BM, Olsson C, Factor SM, Moore RL *et al*. Cardiac troponin T mutations result in allele-specific phenotypes in a mouse model for hypertrophic cardiomyopathy. *J Clin Invest* 1999; **104**:469–481.
18. Chandra M, Rundell VL, Tardiff JC, Leinwand LA, De Tombe PP, Solaro RJ. Ca^{2+} activation of myofilaments from transgenic mouse hearts expressing R92Q mutant cardiac troponin T. *Am J Physiol Heart Circ Physiol* 2001;**280**:H705–H713.
19. Chandra M, Tschirgi ML, Tardiff JC. Increase in tension-dependent ATP consumption induced by cardiac troponin T mutation. *Am J Physiol Heart Circ Physiol* 2005;**289**:H2112–H2119.
20. Kamisago M, Sharma SD, DePalma SR, Solomon S, Sharma P, McDonough B *et al*. Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy. *N Engl J Med* 2000;**343**:1688–1696.
21. Morimoto S, Lu QW, Harada K, Takahashi-Yanaga F, Minakami R, Ohta M *et al*. Ca^{2+}-desensitizing effect of a deletion mutation ΔK210 in cardiac troponin T that causes familial dilated cardiomyopathy. *Proc Natl Acad Sci USA* 2002;**99**:913–918.
22. Robinson P, Mirza M, Knott A, Abdulrazzak H, Willott R, Marston S *et al*. Alterations in thin filament regulation induced by a human cardiac troponin T mutant that causes dilated cardiomyopathy are distinct from

those induced by troponin T mutants that cause hypertrophic cardiomyopathy. *J Biol Chem* 2002;**277**:40710–40716.
23. Venkatraman G, Harada K, Gomes AV, Kerrick WG, Potter JD. Different functional properties of troponin T mutants that cause dilated cardiomyopathy. *J Biol Chem* 2003;**278**:41670–41676.
24. Li D, Czernuszewicz GZ, Gonzalez O, Tapscott T, Karibe A, Durand J-B *et al*. Novel cardiac troponin t mutation as a cause of familial dilated cardiomyopathy. *Circulation* 2001;**104**:2188–2193.
25. Lu QW, Morimoto S, Harada K, Du CK, Takahashi-Yanaga F, Miwa Y *et al*. Cardiac troponin T mutation R141W found in dilated cardiomyopathy stabilizes the troponin T-tropomyosin interaction and causes a Ca^{2+} desensitization. *J Mol Cell Cardiol* 2003;**35**:1421–1427.
26. Mogensen J, Murphy RT, Shaw T, Bahl A, Redwood C, Watkins H *et al*. Severe disease expression of cardiac troponin C and T mutations in patients with idiopathic dilated cardiomyopathy. *J Am Coll Cardiol* 2004;**44**:2033–2040.
27. Mirza M, Marston S, Willott R, Ashley C, Mogensen J, McKenna W *et al*. Dilated cardiomyopathy mutations in three thin filament regulatory proteins result in a common functional phenotype. *J Biol Chem* 2005;**280**:28498–28506.
28. Du C-K, Morimoto S, Nishii K, Minakami R, Ohta M, Tadano N *et al*. Knock-in mouse model of dilated cardiomyopathy caused by troponin mutation. *Circ Res* 2007;**101**:185–194.
29. Kimura A, Harada H, Park JE, Nishi H, Satoh M, Takahashi M *et al*. Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy. *Nat Genet* 1997;**16**:379–382.
30. Takahashi-Yanaga F, Morimoto S, Harada K, Minakami R, Shiraishi F, Ohta M *et al*. Functional consequences of the mutations in human cardiac troponin I gene found in familial hypertrophic cardiomyopathy. *J Mol Cell Cardiol* 2001;**33**:2095–2107.
31. James J, Zhang Y, Osinska H, Sanbe A, Klevitsky R, Hewett TE *et al*. Transgenic modeling of a cardiac troponin I mutation linked to familial hypertrophic cardiomyopathy. *Circ Res* 2000;**87**:805–811.
32. Murphy RT, Mogensen J, Shaw A, Kubo T, Hughes S, McKenna WJ. Novel mutation in cardiac troponin I in recessive idiopathic dilated cardiomyopathy. *The Lancet* 2004;**363**:371–372.
33. Mogensen J, Kubo T, Duque M, Uribe W, Shaw A, Murphy R *et al*. Idiopathic restrictive cardiomyopathy is part of the clinical expression of cardiac troponin I mutations. *J Clin Invest* 2003;**111**:209–216.
34. Gomes AV, Liang J, Potter JD. Mutations in human cardiac troponin I that are associated with restrictive cardiomyopathy affect basal ATPase activity and the calcium sensitivity of force development. *J Biol Chem* 2005;**280**:30909–30915.
35. Yumoto F, Lu QW, Morimoto S, Tanaka H, Kono N, Nagata K *et al*. Drastic Ca^{2+} sensitization of myofilament associated with a small structural change in troponin I in inherited restrictive cardiomyopathy. *Biochem Biophys Res Commun* 2005;**338**:1519–1526.
36. Hoffmann B, Schmidt-Traub H, Perrot A, Osterziel KJ, Gessner R. First mutation in cardiac troponin C, L29Q, in a patient with hypertrophic cardiomyopathy. *Hum Mutat* 2001;**17**:524.
37. Finley N, Abbott MB, Abusamhadneh E, Gaponenko V, Dong W, Gasmi-Seabrook G *et al*. NMR analysis of cardiac troponin C-troponin I complexes: effects of phosphorylation. *FEBS Lett* 1999;**453**:107–112.
38. Schmidtmann A, Lindow C, Villard S, Heuser A, Mugge A, Gessner R *et al*. Cardiac troponin C-L29Q, related to hypertrophic cardiomyopathy, hinders the transduction of the protein kinase A dependent phosphorylation signal from cardiac troponin I to C. *Febs J* 2005;**272**:6087–6097.
39. Preston LC, Lipscomb S, Robinson P, Mogensen J, McKenna WJ, Watkins H *et al*. Functional effects of the DCM mutant Gly159Asp troponin C in skinned muscle fibres. *Pflugers Arch* 2007;**453**:771–776.
40. Preston LC, Ashley CC, Redwood CS. DCM troponin C mutant Gly159Asp blunts the response to troponin phosphorylation. *Biochem Biophys Res Commun* 2007;**360**:27–32.
41. Biesiadecki BJ, Kobayashi T, Walker JS, Solaro RJ, de Tombe PP. The troponin C G159D mutation blunts myofilament desensitization induced by troponin I Ser23/24 phosphorylation. *Circ Res* 2007;**100**:1486–1493.
42. Jaaskelainen P, Miettinen R, Karkkainen P, Toivonen L, Laakso M, Kuusisto J. Genetics of hypertrophic cardiomyopathy in eastern Finland: few founder mutations with benign or intermediary phenotypes. *Ann Med* 2004;**36**:23–32.
43. Chang AN, Harada K, Ackerman MJ, Potter JD. Functional consequences of hypertrophic and dilated cardiomyopathy-causing mutations in α-tropomyosin. *J Biol Chem* 2005;**280**:34343–34349.
44. Heller MJ, Nili M, Homsher E, Tobacman LS. Cardiomyopathic tropomyosin mutations that increase thin filament Ca^{2+} sensitivity and tropomyosin N-domain flexibility. *J Biol Chem* 2003;**278**:41742–41748.
45. Karibe A, Tobacman LS, Strand J, Butters C, Back N, Bachinski LL *et al*. Hypertrophic cardiomyopathy caused by a novel alpha-tropomyosin mutation (V95A) is associated with mild cardiac phenotype, abnormal calcium binding to troponin, abnormal myosin cycling, and poor prognosis. *Circulation* 2001;**103**:65–71.
46. Coviello DA, Maron BJ, Spirito P, Watkins H, Vosberg HP, Thierfelder L *et al*. Clinical features of hypertrophic cardiomyopathy caused by mutation of a 'hot spot' in the α-tropomyosin gene. *J Am Coll Cardiol* 1997;**29**:635–640.
47. Yamauchi-Takihara K, Nakajima-Taniguchi C, Matsui H, Fujio Y, Kunisada K, Nagata S *et al*. Clinical implications of hypertrophic cardiomyopathy associated with mutations in the α-tropomyosin gene. *Heart* 1996;**76**:63–65.
48. Bottinelli R, Coviello DA, Redwood CS, Pellegrino MA, Maron BJ, Spirito P *et al*. A mutant tropomyosin that causes hypertrophic cardiomyopathy is expressed in vivo and associated with an increased calcium sensitivity. *Circ Res* 1998;**82**:106–115.
49. Muthuchamy M, Pieples K, Rethinasamy P, Hoit B, Grupp IL, Boivin GP *et al*. Mouse model of a familial hypertrophic cardiomyopathy mutation in α-tropomyosin manifests cardiac dysfunction. *Circ Res* 1999;**85**:47–56.
50. Prabhakar R, Boivin GP, Grupp IL, Hoit B, Arteaga G, Solaro RJ *et al*. A familial hypertrophic cardiomyopathy α-tropomyosin mutation causes severe cardiac hypertrophy and death in mice. *J Mol Cell Cardiol* 2001;**33**:1815–1828.
51. Michele DE, Gomez CA, Hong KE, Westfall MV, Metzger JM. Cardiac dysfunction in hypertrophic cardiomyopathy mutant tropomyosin mice is transgene-dependent, hypertrophy-independent, and improved by β-blockade. *Circ Res* 2002;**91**:255–262.
52. Thierfelder L, Watkins H, MacRae C, Lamas R, McKenna W, Vosberg HP *et al*. α-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. *Cell* 1994;**77**:701–712.
53. Olson TM, Kishimoto NY, Whitby FG, Michels VV. Mutations that alter the surface charge of α-tropomyosin are associated with dilated cardiomyopathy. *J Mol Cell Cardiol* 2001;**33**:723–732.
54. Rajan S, Ahmed RP, Jagatheesan G, Petrashevskaya N, Boivin GP, Urboniene D *et al*. Dilated cardiomyopathy mutant tropomyosin mice develop cardiac dysfunction with significantly decreased fractional shortening and myofilament calcium sensitivity. *Circ Res* 2007;**101**:205–214.
55. Carrier L, Boheler KR, Chassagne C, de la Bastie D, Wisnewsky C, Lakatta EG *et al*. Expression of the sarcomeric actin isogenes in the rat heart with development and senescence. *Circ Res* 1992;**70**:999–1005.
56. Ilkovski B, Clement S, Sewry C, North KN, Cooper ST. Defining alpha-skeletal and alpha-cardiac actin expression in human heart and skeletal muscle explains the absence of cardiac involvement in ACTA1 nemaline myopathy. *Neuromuscul Disord* 2005;**15**:829–835.
57. Wong WW, Doyle TC, Cheung P, Olson TM, Reisler E. Functional studies of yeast actin mutants corresponding to human cardiomyopathy mutations. *J Muscle Res Cell Motil* 2001;**22**:665–674.
58. Vang S, Corydon TJ, Borglum AD, Scott MD, Frydman J, Mogensen J *et al*. Actin mutations in hypertrophic and dilated cardiomyopathy cause inefficient protein folding and perturbed filament formation. *Febs J* 2005;**272**:2037–2049.
59. Bookwalter CS, Trybus KM. Functional consequences of a mutation in an expressed human α-cardiac actin at a site implicated in familial hypertrophic cardiomyopathy. *J Biol Chem* 2006;**281**:16777–16784.
60. Olson TM, Michels VV, Thibodeau SN, Tai YS, Keating MT. Actin mutations in dilated cardiomyopathy, a heritable form of heart failure. *Science* 1998;**280**:750–752.
61. Lorenz M, Poole KJ, Popp D, Rosenbaum G, Holmes KC. An atomic model of the unregulated thin filament obtained by X-ray fiber diffraction on oriented actin-tropomyosin gels. *J Mol Biol* 1995;**246**:108–119.
62. Lebart MC, Mejean C, Boyer M, Roustan C, Benyamin Y. Localization of a new α-actinin binding site in the COOH-terminal part of actin sequence. *Biochem Biophys Res Commun* 1990;**173**:120–126.
63. Siedner S, Kruger M, Schroeter M, Metzler D, Roell W, Fleischmann BK *et al*. Developmental changes in contractility and sarcomeric proteins from the early embryonic to the adult stage in the mouse heart. *J Physiol* 2003;**548**:493–505.
64. Epstein ND, Cohn GM, Cyran F, Fananapazir L. Differences in clinical expression of hypertrophic cardiomyopathy associated with two distinct mutations in the β-myosin heavy chain gene. *Circulation* 1992;**86**:345–352.

65. Watkins H, Rosenzweig A, Hwang DS, Levi T, McKenna W, Seidman CE *et al*. Characteristics and prognostic implications of myosin missense mutations in familial hypertrophic cardiomyopathy. *N Engl J Med* 1992;**326**:1108–1114.
66. Geisterfer-Lowrance AA, Christe M, Conner DA, Ingwall JS, Schoen FJ, Seidman CE *et al*. A mouse model of familial hypertrophic cardiomyopathy. *Science* 1996;**272**:731–734.
67. Georgakopoulos D, Christe ME, Giewat M, Seidman CM, Seidman JG, Kass DA. The pathogenesis of familial hypertrophic cardiomyopathy: early and evolving effects from an α-cardiac myosin heavy chain missense mutation. *Nat Med* 1999;**5**:327–330.
68. Gao WD, Perez NG, Seidman CE, Seidman JG, Marban E. Altered cardiac excitation-contraction coupling in mutant mice with familial hypertrophic cardiomyopathy. *J Clin Invest* 1999;**103**:661–666.
69. Tyska MJ, Hayes E, Giewat M, Seidman CE, Seidman JG, Warshaw DM. Single-molecule mechanics of R403Q cardiac myosin isolated from the mouse model of familial hypertrophic cardiomyopathy. *Circ Res* 2000;**86**:737–744.
70. Debold EP, Schmitt JP, Patlak JB, Beck SE, Moore JR, Seidman JG *et al*. Hypertrophic and dilated cardiomyopathy mutations differentially affect the molecular force generation of mouse α-cardiac myosin in the laser trap assay. *Am J Physiol Heart Circ Physiol* 2007;**293**:H284–H291.
71. Lowey S. Functional consequences of mutations in the myosin heavy chain at sites implicated in familial hypertrophic cardiomyopathy. *Trends Cardiovasc Med* 2002;**12**:348–354.
72. Marian AJ, Wu Y, Lim DS, McCluggage M, Youker K, Yu QT *et al*. A transgenic rabbit model for human hypertrophic cardiomyopathy. *J Clin Invest* 1999;**104**:1683–1692.
73. Schmitt JP, Debold EP, Ahmad F, Armstrong A, Frederico A, Conner DA *et al*. Cardiac myosin missense mutations cause dilated cardiomyopathy in mouse models and depress molecular motor function. *Proc Natl Acad Sci USA* 2006;**103**:14525–14530.
74. Poetter K, Jiang H, Hassanzadeh S, Master SR, Chang A, Dalakas MC *et al*. Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. *Nat Genet* 1996;**13**:63–69.
75. Vemuri R, Lankford EB, Poetter K, Hassanzadeh S, Takeda K, Yu ZX *et al*. The stretch-activation response may be critical to the proper functioning of the mammalian heart. *Proc Natl Acad Sci USA* 1999;**96**:1048–1053.
76. Sanbe A, Nelson D, Gulick J, Setser E, Osinska H, Wang X *et al*. In vivo analysis of an essential myosin light chain mutation linked to familial hypertrophic cardiomyopathy. *Circ Res* 2000;**87**:296–302.
77. James J, Zhang Y, Wright K, Witt S, Glascock E, Osinska H *et al*. Transgenic rabbits expressing mutant essential light chain do not develop hypertrophic cardiomyopathy. *J Mol Cell Cardiol* 2002;**34**:873–882.
78. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C *et al*. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation* 2003;**107**:2227–2232.
79. Szczesna-Cordary D, Guzman G, Zhao J, Hernandez O, Wei J, Diaz-Perez Z. The E22K mutation of myosin RLC that causes familial hypertrophic cardiomyopathy increases calcium sensitivity of force and ATPase in transgenic mice. *J Cell Sci* 2005;**118**:3675–3683.
80. Dumka D, Talent J, Akopova I, Guzman G, Szczesna-Cordary D, Borejdo J. E22K mutation of RLC that causes familial hypertrophic cardiomyopathy in heterozygous mouse myocardium: effect on cross-bridge kinetics. *Am J Physiol Heart Circ Physiol* 2006;**291**:H2098–H2106.
81. Niimura H, Patton KK, McKenna WJ, Soults J, Maron BJ, Seidman JG *et al*. Sarcomere protein gene mutations in hypertrophic cardiomyopathy of the elderly. *Circulation* 2002;**105**:446–451.
82. Yang Q, Sanbe A, Osinska H, Hewett TE, Klevitsky R, Robbins J. A mouse model of myosin binding protein C human familial hypertrophic cardiomyopathy. *J Clin Invest* 1998;**102**:1292–1300.
83. Harris SP, Bartley CR, Hacker TA, McDonald KS, Douglas PS, Greaser ML *et al*. Hypertrophic cardiomyopathy in cardiac myosin binding protein-C knockout mice. *Circ Res* 2002;**90**:594–601.
84. Itoh-Satoh M, Hayashi T, Nishi H, Koga Y, Arimura T, Koyanagi T *et al*. Titin mutations as the molecular basis for dilated cardiomyopathy. *Biochem Biophys Res Commun* 2002;**291**:385–393.
85. Satoh M, Takahashi M, Sakamoto T, Hiroe M, Marumo F, Kimura A. Structural analysis of the titin gene in hypertrophic cardiomyopathy: identification of a novel disease gene. *Biochem Biophys Res Commun* 1999;**262**:411–417.
86. Matsumoto Y, Hayashi T, Inagaki N, Takahashi M, Hiroi S, Nakamura T *et al*. Functional analysis of titin/connectin N2-B mutations found in cardiomyopathy. *J Muscle Res Cell Motil* 2005;**26**:367–374.
87. Lange S, Auerbach D, McLoughlin P, Perriard E, Schafer BW, Perriard J-C *et al*. Subcellular targeting of metabolic enzymes to titin in heart muscle may be mediated by DRAL/FHL-2. *J Cell Sci* 2002;**115**:4925–4936.
88. Knoll R, Hoshijima M, Hoffman HM, Person V, Lorenzen-Schmidt I, Bang ML *et al*. The cardiac mechanical stretch sensor machinery involves a Z disc complex that is defective in a subset of human dilated cardiomyopathy. *Cell* 2002;**111**:943–955.
89. Bos JM, Poley RN, Ny M, Tester DJ, Xu X, Vatta M *et al*. Genotype-phenotype relationships involving hypertrophic cardiomyopathy-associated mutations in titin, muscle LIM protein, and telethonin. *Mol Genet Metab* 2006;**88**:78–85.
90. Hayashi T, Arimura T, Itoh-Satoh M, Ueda K, Hohda S, Inagaki N *et al*. Tcap gene mutations in hypertrophic cardiomyopathy and dilated cardiomyopathy. *J Am Coll Cardiol* 2004;**44**:2192–2201.
91. Geier C, Perrot A, Ozcelik C, Binner P, Counsell D, Hoffmann K *et al*. Mutations in the human muscle LIM protein gene in families with hypertrophic cardiomyopathy. *Circulation* 2003;**107**:1390–1395.
92. Mohapatra B, Jimenez S, Lin JH, Bowles KR, Coveler KJ, Marx JG *et al*. Mutations in the muscle LIM protein and α-actinin-2 genes in dilated cardiomyopathy and endocardial fibroelastosis. *Mol Genet Metab* 2003;**80**:207–215.
93. Osio A, Tan L, Chen SN, Lombardi R, Nagueh SF, Shete S *et al*. Myozenin 2 is a novel gene for human hypertrophic cardiomyopathy. *Circ Res* 2007;**100**:766–768.
94. Arimura T, Hayashi T, Matsumoto Y, Shibata H, Hiroi S, Nakamura T *et al*. Structural analysis of four and half LIM protein-2 in dilated cardiomyopathy. *Biochem Biophys Res Commun* 2007;**357**:162–167.
95. Vatta M, Mohapatra B, Jimenez S, Sanchez X, Faulkner G, Perles Z *et al*. Mutations in Cypher/ZASP in patients with dilated cardiomyopathy and left ventricular non-compaction. *J Am Coll Cardiol* 2003;**42**:2014–2027.
96. Arimura T, Hayashi T, Terada H, Lee SY, Zhou Q, Takahashi M *et al*. A Cypher/ZASP mutation associated with dilated cardiomyopathy alters the binding affinity to protein kinase C. *J Biol Chem* 2004;**279**:6746–6752.
97. Javadpour MM, Tardiff JC, Pinz I, Ingwall JS. Decreased energetics in murine hearts bearing the R92Q mutation in cardiac troponin T. *J Clin Invest* 2003;**112**:768–775.
98. Spindler M, Saupe KW, Christe ME, Sweeney HL, Seidman CE, Seidman JG *et al*. Diastolic dysfunction and altered energetics in the $\alpha MHC^{403/+}$ mouse model of familial hypertrophic cardiomyopathy. *J Clin Invest* 1998;**101**:1775–1783.
99. Crilley JG, Boehm EA, Blair E, Rajagopalan B, Blamire AM, Styles P *et al*. Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy. *J Am Coll Cardiol* 2003;**41**:1776–1782.
100. Ashrafian H, Redwood C, Blair E, Watkins H. Hypertrophic cardiomyopathy:a paradigm for myocardial energy depletion. *Trends Genet* 2003;**19**:263–268.