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Rescue of Familial Cardiomyopathies by Modifications at the Level of Sarcomere and Ca^{2+} Fluxes

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Abstract

Cardiomypathies are a heterogeneous group of diseases of the myocardium associated with mechanical and/or electrical dysfunction that frequently show inappropriate ventricular hypertrophy or dilation. Current data suggest that numerous mutations in several genes can cause cardiomyopathies, and the severity of their phenotypes is also influenced by modifier genes. Two major types of inherited cardiomyopathies include familial hypertrophic cardiomyopathy (FHC) and dilated cardiomyopathy (DCM). FHC typically involves increased myofilament Ca^{2+} sensitivity associated with diastolic dysfunction, whereas DCM often results in decreased myofilament Ca^{2+} sensitivity and systolic dysfunction. Besides alterations in myofilament Ca^{2+} sensitivity, alterations in the levels of Ca^{2+}-handling proteins have also been described in both diseases. Recent work in animal models has attempted to rescue FHC and DCM via modifications at the myofilament level, altering Ca^{2+} homeostasis by targeting Ca^{2+}-handling proteins, such as the sarcoplasmic reticulum ATPase and phospholamban, or by interfering with the products of different modifiers genes. Although attempts to rescue cardiomyopathies in animal models have shown great promise, further studies are needed to validate these strategies in order to provide more effective and specific treatments.

INTRODUCTION

The term “cardiomyopathy” was first used in 1957 and since then the knowledge about this group of complex cardiac diseases has increased substantially. Concomitant with this increasing knowledge has been changes in the classification of cardiomyopathies. Currently, the American Heart Association has adopted the following definition proposed in 2006: “Cardiomyopathies are a heterogeneous group of diseases of the myocardium associated with mechanical and/or electrical dysfunction that usually (but not invariably) exhibit inappropriate ventricular hypertrophy or dilatation and are due to a variety of causes that frequently are genetic. Cardiomyopathies either are confined to the heart or are part of generalized systemic disorders, often leading to cardiovascular death or progressive heart failure-related disability” [1].

Cardiomyopathies can be divided into two groups: 1) primary and 2) secondary. Primary cardiomyopathies describe diseases in which the heart is the sole or predominantly organ

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involved, while secondary cardiomyopathies describe those in which cardiac function is impaired due to systemic disorders [2]. Primary cardiomyopathies can be subdivided into three groups: a) genetic cardiomyopathies: familial hypertrophic cardiomyopathy (FHC), arrhythmogenic right ventricular cardiomyopathy/dysplasia, left ventricular noncompaction, glycogen storage cardiomyopathies, conduction system disease cardiomyopathies, mitochondrial cardiomyopathies and ion channel-related cardiomyopathies; b) mixed (genetic and nongenetic): dilated cardiomyopathy (DCM) and restrictive cardiomyopathy; and c) acquired: inflammatory, stress-provoked, peripartum, tachycardia-induced and infants of insulin-dependent diabetic mothers [1].

Recent work has done much to identify the genes involved in cardiomyopathies. However, the molecular steps which connect gene defects to clinical phenotypes are still unknown. Genetic and molecular biology studies have provided new insights into the pathophysiology of the cardiomyopathies, and are now beginning to have an impact in guiding preventive and therapeutic strategies for these diseases. The current article focuses mainly on genetic cardiomyopathies linked to sarcomeric proteins. We review the recent advances in experimental pharmacological and molecular strategies for treatment of cardiomyopathies with emphasis on interventions affecting calcium handling and sarcomeric proteins.

HYPERTROPHIC CARDIOMYOPATHY

Hypertrophic cardiomyopathy is characterized by unexplained left ventricle hypertrophy, having an overall prevalence of 200 per 100,000 individuals [2]. The genetic form of the disease, referred to as familial hypertrophic cardiomyopathy (FHC), is inherited as an autosomal trait and has been linked to mutations in sarcomeric protein genes in the vast majority of cases, although phenocopies have been observed in metabolic, mitochondrial and neuromuscular cardiomyopathies [1]. To date, over 400 FHC-causing mutations (see Table 1) in different components of the sarcomere have been reported reflecting its marked genetic heterogeneity [3]. Sarcomere-linked mutations account for about up to 65% of all diagnosed cases of FHC [4]. The main genes affected are *MYH7* (beta myosin heavy chain or β-MyHC), *MYBPC3* (myosin binding protein C or MyBPC), *TNNT2* (cardiac troponin T or cTnT), *TNNI3* (cardiac troponin I or cTnI), *TPM1* (alpha tropomyosin or α-Tm), *MYL2* (regulatory myosin light chain or RLC), *MYL3* (essential myosin light chain or ELC), *TNNC1* (cardiac troponin C or cTnC), *ACTC1* (alpha cardiac actin or α-actin) and *TTN* (titin) (see Table 1).

FHC is largely identified by the presence of unexplained left ventricle (LV) hypertrophy together with other echocardiographic and histopathological features such as LV outflow tract obstruction, diastolic dysfunction with preserved ejection fraction and increased interstitial fibrosis with myocyte hypertrophy/disarray [5]. The diversity of causal mutations, associated with a variable genetic background and the influence of modifier genes, leads to a wide variability in FHC-phenotypic expression [6–8]. FHC follows a variable clinical course, can be diagnosed at any age and manifests itself across a wild spectrum spanning from mild cardiac hypertrophy and no symptoms to marked hypertrophy with diastolic heart failure and sudden death [9].

The current medical therapy of FHC aims to relieve symptoms and includes the use of β-blockers, the Ca^{2+} channel blocker verapamil, and the Na^{+} channel blocker disopyramide [10–12]. In drug refractory patients, the therapeutic options are surgical myectomy, alcohol septal ablation, dual-chamber (DDD) pacing and heart transplantation [13–15]. Although the overall survival of patients with FHC is similar to the general population, the risk of sudden death is increased, especially in young people and athletes, and is often the first manifestation of the disease.

DILATED CARDIOMYOPATHY

DCM is characterized by enlargement of the cardiac chambers, decreased myocardial contractility and unspecific histopathological findings, such as myocyte loss, increased apoptosis and interstitial fibrosis [1,16]. DCM is an important cause of cardiac morbidity and mortality and the leading cause of cardiac transplantation, with an estimated prevalence of 36.5 per 100,000 individuals. It has been linked to genetic causes in approximately 25–30% of the cases [17,18]. Autosomal dominance is the most commonly observed pattern of inheritance, but X-linked, autosomal recessive and mitochondrial DNA mutations (matrilinear inheritance) also occur [1]. DCM was initially identified in genes coding for proteins of the cytoskeleton and Z-disc [2]. Thus, it has been described as a disease resulting from impaired force "transmission", due to the role of these proteins in translating force generated by the sarcomere to the extracellular matrix [19,20]. However, a significant number of mutations in sarcomeric proteins that lead to disruption of sarcomere activation have also been demonstrated, thereby implicating impaired force "generation" as an additional mechanism in the pathogenesis of the disease [18,21,22]. Gene mutations linked to DCM are listed in Table 1.

As with FHC, the clinical presentation of DCM is widely variable, ranging from an asymptomatic life-long course to rapid and progressive heart failure requiring cardiac transplantation [17,23]. The diagnosis of asymptomatic patients can be incidental in routine medical screening or after family evaluation of patients with established diagnosis. More typically, however, patients present at the time of diagnosis with symptoms of pulmonary congestion or low cardiac output [17]. DCM is often associated with defects in the conduction system, arrhythmias and sudden death that have been linked to myocardial remodeling and increased fibrosis. Medical treatment includes combined use of angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor (AR) antagonists, β-blockers, aldosterone antagonists and diuretics [16]. Despite new advances in the treatment of patients with DCM in the last decade, mortality is still elevated, approaching 50% at five years in symptomatic patients [24].

GENETIC AND MOLECULAR PATHOGENESIS

Defective proteins resulting from genetic mutations can lead to a disruption of the mechanisms of force production and transmission, resulting in impaired cardiomyocyte contractility and relaxation. However, the mechanism by which a single mutation leads to a specific pathological phenotype, the signaling pathways activated to determine that phenotype, and the role of other genetic and environmental factors that influence the phenotype remain poorly understood. In the past two decades, several studies have demonstrated that genetic FHC and DCM most commonly result from defects in genes encoding proteins of the sarcomere and Z-disc, but also involve defective proteins of the cytoskeleton/sarcolemma, sarcoplasmic reticulum, nuclear membrane, intercalated disc, and altered metabolic and transcriptional pathways (Table 1).

Thick filament mutations

The thick filament consists of myosin associated with the essential (ELC) and regulatory (RLC) light chains. Mutations in β-MyHC account for 20–30% of all FHC patients, and they are also an important cause of DCM [6,18,22,25]. In general, FHC linked to mutations in β-MyHC lead to a clinical phenotype characterized by severe hypertrophy and high risk of sudden death, with an early onset and poor prognosis [25]. Mutations such as R453Q and R1053G are associated with an FHC phenotype that gradually transitions to DCM, while others such as S532P and F764L result in primary DCM [22,26]. Thus, different mutations in the same molecule can lead to diverse phenotypes.

Most of these mutations occur in the globular head or near the head-rod junction of the myosin molecule. The missense mutation R403Q in β-MyHC is located at the base of the surface loop that attaches the myosin head to actin, and was the first to be linked to FHC. Therefore, it is intuitive to hypothesize that a resulting effect of the mutation would be to disrupt the actomyosin interaction. Indeed, initial *in vitro* studies have described functional abnormalities caused by the R403Q mutation, including decreased actin-activated ATPase activity and reduced actin sliding speed [27–29]. These results suggested that the hypertrophic response observed in R403Q carriers could represent a compensation for decreased force generation. However, other studies using purified myosin or skinned cardiac fibers from TG mice expressing the R403Q mutation have shown increased actin-dependent ATPase, actin sliding speed [30,31] and Ca^{2+} sensitivity [32,33]. These results suggest that instead of decreasing the power generation, the R403Q mutation actually potentiates it and thereby leads to "gain of function". Debold *et al.* [34] have also shown that the FHC-linked mutations R403Q and R453Q increase the force generation per cross bridge in the laser trap assay, while the DCM-linked mutations S532P and F764L show a decrease.

In addition to gain of function, Semsariam *et al.* [35] have hypothesized that altered biophysical properties of the R403Q mutation lead to Ca^{2+} retention by the myofilament (Ca^{2+} trapping). According to this hypothesis, increased myofilament Ca^{2+} affinity would lead to decreased kinetics of relaxation, which is compatible with the clinical observation that diastolic dysfunction is the primary defect in FHC hearts. Besides the abnormalities of the myofilament, they also suggested that "abnormal SR Ca^{2+} responses and reduced Ca^{2+}-binding proteins are early events in the pathogenesis of hypertrophic cardiomyopathy". In their study, α-MyHC R403Q mice exhibit decreased SR Ca^{2+} content, decreased calsequestrin and ryanodine receptor (RyR2) expression, and increased RyR2 phosphorylation. Although the Ca^{2+} trapping hypothesis is attractive, further data are necessary to confirm it.

Finally, Spindler *et al.* [36] have shown that α-MyHC R403Q mice have altered myocardial energetics, as demonstrated by ^{31}P NMR spectroscopy studies. These studies showed that α-MyHC R403Q mice had decreased phosphocreatine (PCr), increased inorganic phosphate (Pi) and a decreased calculated free energy release from ATP hydrolysis, when compared to wild type mice. The authors hypothesized that the free energy available during times of high energy consumption in α-MyHC R403Q hearts would not be enough to maintain the cytoplasmic-SR Ca^{2+} gradient, which could result in diastolic Ca^{2+} overload. They also suggested that the energetic abnormalities in α-MyHC R403Q are likely to be primarily caused by the myosin mutation, with less cross-bridge produced force per ATP hydrolysed, and are not secondary to hypertrophy. This hypothesis has been supported by ^{31}P NMR spectroscopy studies in patients with FHC expressing mutations in β-MyHC (16 patients), TnT (8 patients) and MyBP-C (7 patients) in which a decreased PCr to ATP ratio in human FHC hearts was observed irrespective of the degree of hypertrophy [37].

Thin filament mutations

Functional units of thin filaments consist of seven actin monomers, one coiled-coil Tm protein and one Tn complex, which itself is comprised of three units: TnT, TnI and TnC. The thin filament plays an important role in muscle contraction by translating the Ca^{2+} signal into sarcomere activation and force production following a complex sequence of protein-protein interactions. In systole, Ca^{2+} binds to the regulatory site on cTnC and brings about conformational alterations in the Tn complex, which in turn shifts the position of Tm on the actin molecule and exposes its myosin binding sites. Activation of the actomyosin complex results in sliding of the thin filament along the thick filament, sarcomere shortening and muscle contraction [38].

It has been demonstrated that modification of this finely tuned mechanism by mutations in thin filament components can lead to the development of FHC and DCM [6,39]. Indeed, studies have revealed that mutations in the *TNNT2*, *TPM1*, *TNNI3*, *ACTC1* and *TNNC1* genes are linked to the pathogenesis of FHC or DCM [22,40]. Thus, any component of the thin filament can be affected, and the development of FHC or DCM phenotypes is dependent on the specific mutation and other genetic and non-genetic modifier factors [7].

The clinical phenotypes associated with TnT mutations have been extensively described [41, 42], whereas phenotypes from mutations in Tm, TnI, TnC and actin are less well-characterized in humans due to the limited number of genotype-phenotype studies [43–45]. Taken together, the phenotypes of thin filament-linked cardiomyopathies are quite heterogeneous and include families with a relatively benign course and others with severe or malignant cardiomyopathies. One remarkable characteristic that "defines" thin filament-linked FHC is the apparent dissociation between the degree of hypertrophy and the clinical outcome. On average, thin filament mutations result in less hypertrophy and cardiac remodeling when compared to β-MyHC mutations [40]. However, mutations in TnT have been associated with a high incidence of sudden death and poor prognosis, which implies an associated arrhythmic cellular mechanism, even in the absence of hypertrophy and fibrosis [46].

Several studies have evaluated the effects of thin filament mutations on the mechanism of Ca^{2+}-dependent activation of the myofilament. Most of the studies have shown that virtually all mutations in TnT, Tm and TnI can modify the myofilament response to Ca^{2+} [47]. Moreover, different groups have shown that thin filament FHC-linked mutations increase myofilament Ca^{2+} sensitivity and lead to diastolic dysfunction while DCM-linked mutations have the opposite effect, resulting in systolic dysfunction [48–55] [56,57] [52,58,59,59–61]. Michele *et al.* [62] have also demonstrated that different FHC-linked mutations in thin filaments fit into a specific hierarchy in their capacity to increase the Ca^{2+} sensitivity, and that the magnitude of this increase is transgene dose-dependent.

It has been proposed that FHC- and DCM-causing mutations in Tn and Tm result in altered flexibility of these proteins, which might modify their interaction and consequently alter Ca^{2+}-dependent tension development. This hypothesis has been supported by recent work in which the authors used a fluorescent probe to measure the Ca^{2+}-binding affinity to TnC in order to determine the effect of different mutations in the reconstituted thin filament [63,64]. They have shown that FHC- or DCM-linked mutations in thin filament proteins alter the Ca^{2+}-binding affinity of TnC only when incorporated into the fully integrated thin filament, suggesting that the mutations lead to a disruption in thin filament cooperative activation.

EXPERIMENTAL STRATEGIES TO RESCUE CARDIOMYOPATHIES

Targeting the myofilaments

Although mutations in sarcomeric proteins lead to a wide spectrum of cardiomyopathic phenotypes and result from an array of factors, the primary defect of FHC and DCM lies in altered myofilament properties. Functionally, the major defect and common thread in DCM is systolic dysfunction often associated with decreased myofilament Ca^{2+} sensitivity, whereas in FHC the major defect is diastolic dysfunction and in most cases an increase in myofilament Ca^{2+} sensitivity. Examples of this increased myofilament Ca^{2+} sensitivity in animal models of human FHC include Tm [48–50], TnT [51–55] or TnI [56,57]. In contrast, animal models of DCM in Tm [58] and TnT [59,60] show decreased myofilament sensitivity to Ca^{2+}. If the primary defect in sarcomere-linked cardiomyopathies is altered myofilament Ca^{2+} sensitivity, a logical therapeutic approach would be to bring their sensitivity back to normal levels, preferably early in the development of the disease. In heart failure (HF), increasing sarcomeric activity by pharmacological sensitization of the myofilament to Ca^{2+} has provided beneficial

effects in the short term [65–67]. However, little is known as to whether interventions via sarcomeric sensitization to Ca^{2+} might be beneficial in DCM or whether desensitization is beneficial in FHC. In addition, it is not known whether there is a specific time period when the therapy should be initiated. To the best of our knowledge there are no published studies concerning early intervention in children from families with familial cardiomyopathies.

There are several possible targets within the myofilament for altering myofilament Ca^{2+} sensitivity, including both thin filament proteins (TnI, TnC and Tm) and thick filament proteins. An example of the therapeutic potential of myofilament desensitization in FHC was shown in TG mice expressing mutated Tm at position 180 (TmE180G or Tm180) [49]. These mice exhibit increased Ca^{2+} sensitivity and were crossbred with chimeric Tm TG mice with decreased Ca^{2+} sensitivity [68]. The result of this cross produced a mouse with Ca^{2+} sensitivity similar to wild-type, a decrease in both fibrosis and myocyte disarray compared to Tm180, systolic function equivalent to wild-type, and improved diastolic function for up to one year when compared to Tm180 [69].

TnI is also a potential target since its phosphorylation by protein kinase A (PKA), protein kinase C (PKC), protein kinase D (PKD) and p21-activated kinase has significant effects on myofilament properties [70–74]. For example, it is well documented that PKA-mediated phosphorylation of cTnI at residues S23 and S24 results in desensitization of the myofilaments to Ca^{2+} [75,76]. Furthermore, myofilament Ca^{2+} sensitivity is increased in hearts from patients with HF due to reduced level of cTnI phosphorylation [77,78]. In addition, a small amount of myofilament desensitization via exercise following myocardial infarction improved LV function compared to infarcted sedentary mice [79]. Since phosphorylation of TnI at residues S23/S24 decreases myofilament Ca^{2+} sensitivity, this effect could be used as strategy to attenuate the increased Ca^{2+} sensitivity in FHC and early results using this strategy look promising [80]. Collectively, these data suggest interventions that desensitize the myofilament to Ca^{2+} may serve as potential therapies for treating FHC phenotypes associated with increased myofilament Ca^{2+} sensitivity.

If the primary defect of sarcomeric-linked DCM is associated with decreased myofilament Ca^{2+} sensitivity, it would be intuitive that resensitizing the myofilaments to normal levels should be beneficial. To this end, the first class of Ca^{2+} sensitizers was developed almost 20 years ago. Some, such as levosimendan and pimobendan, reached the clinical trial level, but they have not been used for treatment of DCM patients resulting from sarcomeric protein mutations. Levosimendan acts by binding to TnC[81], and it also shows vasodilatory and anti-ischemic effects by opening ATP-sensitive K+ channels in the sarcolemma and mitochondria [82,83]. Thus, levosimendan has two mechanisms of action: increasing inotropism and reducing afterload. In clinically approved doses levosimendan improves cardiac output without impairing relaxation, yet it inhibits phosphodiesterase only at higher doses [84]. So far levosimendan is used only for the treatment of acute and decompensated HF [67]. To our knowledge, it has not been tested in animal models of DCM. On the other hand, pimobendan, which is also used in acute HF, was recently tested in a mouse model of DCM caused by the deletion mutation ΔK210 in TnT[85]. The phenotype of these mice includes cardiac enlargement, reduced cardiac performance and frequent sudden death, while physiological parameters include decreased Ca^{2+} sensitivity that is compensated by increased Ca^{2+} transient amplitude. Early intervention with pimobendan had profound effects on the development of DCM as seen in improvements in cardiac performance and morphology, HF and even sudden cardiac death [85]. Although these results are truly compelling, it would also be interesting to determine if the effects of intervention with pimobendan can reverse the process of DCM-induced cardiac remodeling after full development of the phenotype.

Myofilaments versus calcium-handling proteins

Contraction and relaxation of the heart are regulated by complex processes involving the myofilaments, Ca^{2+}-handling proteins and the loading conditions of the heart (for review see Bers [86]). At the single cardiomyocyte level, the dynamics of contraction and relaxation are regulated both on a beat-to-beat basis (short-term regulation) and as a result of adaptation and maladaptation to different cardiovascular stresses (long-term regulation). In short-term regulation during systole, Ca^{2+} is bound to only 20–25% of troponin C (TnC). Thus, augmented Ca^{2+} delivery to the myofilaments, or increase in their sensitivity to this ion, result in improved contractility. During diastole, extrusion of Ca^{2+} from the cytosol by the sarcoplasmic reticulum Ca^{2+} pump (SERCA2a), the Na^{+}/Ca^{2+} exchanger, and to a marginal extent the sarcolemmal Ca^{2+} pump, returns systolic Ca^{2+} concentration to resting levels and allows for relaxation of the cell [87].

Controversy remains, however, concerning the relative contribution of the myofilaments versus Ca^{2+}-handling proteins to the rate of relaxation in cardiac muscle. Some have argued that relaxation is limited by the myofilaments, since active force in cardiac papillary muscle is maintained for a considerable period after Ca^{2+} concentration returns to resting levels [88]. Others have shown that Ca^{2+} uptake limits the late phase of relaxation in experiments using isolated, unloaded cardiomyocytes [89,90]. This controversy may be partially explained by the differences in experimental conditions such as temperature and loaded versus unloaded preparations. Janssen *et al.* [91] have recently shown that myofilaments may be the rate limiting factor only near physiological temperatures.

Despite the controversy, there is agreement that when the expression of SERCA2a and phospholamban (PLB) are altered such as in HF, Ca^{2+} transient decay significantly contributes to the observed slower relaxation rate (for review see Hasenfuss [92]). To rectify this, studies with PLBKO mice and mice overexpressing SERCA2a have shown that altering Ca^{2+} homeostasis by either method results in a faster rate of relaxation in cells, papillary muscle, and the whole heart. This suggests that it is possible to improve cardiac relaxation by decreasing the decay time of the Ca^{2+} transient [93–97]. The next section discusses recent attempts to rescue HF, FHC and DCM by manipulating sarcoplasmic Ca^{2+}-handling proteins.

Targeting the sarcoplasmic reticulum proteins (SERCA2a and PLB)

In cardiac cells, the SR proteins, SERCA2a and PLB, play a critical role in regulating release and uptake of Ca^{2+} from the SR (for review see Brittsan and Kranias [98]. During diastole, SERCA2a pumps Ca^{2+} from the cytoplasm into the SR, but its activity is inhibited by its association with PLB. Phosphorylation of PLB dissociates it from SERCA2a and thus reverses the inhibition allowing for faster Ca^{2+} reuptake into the SR. The rate of Ca^{2+} uptake by SR also depends on the levels of SERCA2a and PLB protein expression. The PLB/SERCA2a ratio is critical in the regulation of myocardial contractility [99]. The PLB/SERCA2a ratio also affects the force-frequency relationship as myocytes overexpressing SERCA2a exhibit shortened relaxation times and a negative force-frequency relationship, while myocytes overexpressing PLB exhibit prolonged relaxation and an augmented, positive force-frequency relationship [100]. In HF, the SERCA2a/PLB ratio is decreased, reducing the SR Ca^{2+} uptake [101–103]. Thus, because of their importance in the contractile process, modulating levels of either could be beneficial in preventing HF, FHC or DCM. Overexpression of SERCA2a, the muscle-specific isoform, has been used to rescue HF in several instances. In aortic-baned rats, injection of SERCA2a adenovirus into decompensated or failing hearts resulted in normalization of LV systolic pressure, the maximal rate of pressure development and decline, and the rate of isovolumic relaxation (tau) [104]. A similar study showed that SERCA2a adenovirus administered to aortic-banded rats during the HF phase normalized left ventricular volumes and improved both the phosphocreatine/ATP ratio and survival rates [105]. SERCA2a

overexpression also improved function at the cellular level in human ventricular myocytes taken from patients with HF. SERCA2a gene transfer induced faster contraction and relaxation velocities, decreased diastolic Ca^{2+}, increased systolic Ca^{2+} and even normalized the force-frequency relationship [106]. In TG SERCA2a overexpression mice subjected to aortic banding, numerous parameters improved compared to controls including mortality rate, LV systolic function, myocyte fractional shortening and relengthening, calcium transient amplitude and rate of transient decay [107].

While most of these studies have focused on rescuing heart failure resulting from secondary cardiomyopathies, our group has recently shown similar findings in a mouse model of primary FHC, Tm180 [49]. Results indicate that intraventricular injection of SERCA2a adenovirus into one day-old Tm180 mice increased SERCA2a protein expression for several weeks, delayed development of FHC and restored contractile parameters [108,109]. We believe that this is the only study thus far that attempts to rescue FHC via SERCA2a overexpression. Nonetheless, it is notable that depressed cardiac contractility or diastolic dysfunction resulting from reduced SERCA2a expression can be reversed not only by SERCA2a overexpression, but by overexpressing other Ca^{2+}-binding proteins such as sorcin [110] or parvalbumin [111]. Moreover, parvalbumin was shown to correct slower relaxation in adult cardiac cells expressing mutated Tm linked to FHC [112]. Similar to SERCA2a overexpression, decreasing PLB expression has similar beneficial effects on cardiomyopathies. In a mouse model of DCM resulting from ablation of muscle LIM protein [113], crossbreeding with PLBKO mice restored numerous morphological abnormalities including cardiac chamber dilation, myofibrillar disarray and large scale fibrosis [114]. At the cellular level, PLBKO also suprarescued contractile parameters and increased calcium transient amplitude, activation and inactivation kinetics. Interestingly, PLB ablation seems to be dose-dependent as heterozygotes had an intermediate level of rescue. PLBKO was also used to rescue another mouse model of DCM, overexpression of calsequestrin [115]. Calsequestrin TG mice exhibit hypertrophy, increased hypertrophic marker gene expression, reduced levels of LV contraction and relaxation, depressed calcium transient amplitude despite increased SR load, and a decrease in L-type Ca^{2+} channel current [116]. Following crossbreeding with PLBKO mice, these mice displayed increased cardiac performance both *in vivo* and *ex vivo*, decreased inactivation time for L-type Ca^{2+} currents, and reduced expression levels of hypertrophic marker genes [115]. In contrast, in a third mouse model of DCM, tropomodulin TG, crossbreeding with PLBKO mice failed to rescue their dilated phenotype and juvenile lethality [117].

In addition to DCM, PLB ablation has also been used to rescue HF in both failing human myocytes and animal models. In failing human myocytes, gene delivery of an antisense strand to PLB increased contraction and relaxation velocities, enhanced SR Ca^{2+} release and restored the normal frequency response [118]. In a hamster model of HF, *in vivo* gene delivery of pseudophosphorylated PLB increased contractile and relaxation parameters, both in single cardiomyocytes and *in vivo*, and decreased both fibrosis and hypertrophic marker genes. Surprisingly, the effects lasted for 28–30 weeks, giving credence to their newly described method of adenovirus gene delivery [119]. In contrast, PLBKO in the Gαq TG model of HF did not improve hemodynamic parameters, hypertrophy or fibrosis even though unloaded, isolated cardiomyocytes displayed a suprarescue of both fractional shortening and calcium transient amplitude [120].

Finally, PLBKO mice have been used to rescue models of FHC. In the Song *et al.* [120] study listed above, rescue was also attempted in a mouse model of FHC stemming from a mutated form of MyBP-C. Unfortunately, the results were similar to those seen in the G q TG model. Freeman *et al.* [121] also describe attempts to rescue an FHC mutation, MyHC R403Q, via PLBKO. PLBKO increased systolic function and exercise tolerance in the FHC model, yet exacerbated hypertrophy as assessed by heart weight/body weight ratio at 10.5 months. In

contrast, we have recently shown that PLB ablation in a mouse model of FHC, Tm180, provided significant improvement of cardiac function and morphology, including hypertrophy and fibrosis, for up to one year [122].

In addition to alterations in the SR proteins, SERCA2a and PLB, Ca^{2+} fluxes can be modified by using the Ca^{2+} channel blocker diltiazem. Diltiazem has demonstrated efficacy in two different models of FHC [35,123]. Overall, these data strongly suggest that HF, DCM and FHC can be rescued by modifying Ca^{2+} fluxes.

Targeting modifier genes

In addition to incomplete penetrance and diversity of causal mutations in FHC and DCM, studies have shown that the broad heterogeneity in phenotype of these cardiomyopathies is influenced by modifier genes [21,124–127]. Modifier genes compound the individual genetic background that differs within a population due to DNA polymorphism. They are neither necessary nor sufficient to cause disease, but exert an important influence in the expression of a genetic disease. Thus, pharmacological or genetic interventions on target signaling pathways under the influence of modifier genes could result in new strategies of treatment for cardiomyopathies.

Several gene polymorphisms have been considered candidates to modify the phenotype expression of FHC or DCM including ACE, angiotensinogen, AR type 1 and 2, aldosterone synthase, endothelin-1, tumor necrosis factor-α, interleukin-6, insulin-like growth factor-2, transforming growth factor β1, variants of α2c-, β1- and β2-adrenergic receptors and others [126,128–148]. The renin-angiotensin-aldosterone system (RAAS) is one of most widely studied in this context. The role of RAAS in cardiovascular disease such as hypertensive cardiomyopathy, myocardial infarction and HF is well-established [149,150]; however, the impact of polymorphisms in key constituents of RAAS on the severity of phenotype in FHC or DCM has been more controversial [151–154]. For example, the ACE gene has a polymorphic region containing an insertion (I) or deletion (D) of a 287 bp fragment (I/D polymorphism). In some studies, the D/D genotype has been associated with increased hypertrophy and high risk of sudden death in patients with FHC [124,125,132], whereas it correlated with reduced LV systolic performance and increased LV cavity size in patients with DCM [126]. Other studies, in contrast, have shown a lack of association [155,156].

The blockade of key steps in RAAS activation has been correlated with improvement in LV function and cardiac remodeling in rodent models of FHC and DCM by reversing the hypertrophic and profibrotic effects of angiotensin II and aldosterone. ACE inhibition using enalapril alone, or in association with the mineralocorticoid receptor antagonist spironolactone, decreased LV cavity size and collagen density in cardiomyopathic hamsters [157,158]. It has also been shown that aldosterone and aldosterone synthase mRNA levels are elevated in humans with FHC [153]. Aldosterone increases the expression of hypertrophic markers in rat cardiac myocytes through phosphorylation of PKD, and the expression of collagens and transforming growth factor-β1 in rat cardiac fibroblasts through upregulation of phosphoinositide 3- kinase [153]. Spironolactone reversed the hypertrophic and profibrotic effects of aldosterone in a mouse model of human FHC caused by a missense mutation in TnT (R92Q), decreasing interstitial fibrosis, myocyte disarray and improving LV diastolic function [153]. Furthermore, blockade of angiotensin II receptors using losartan also reversed the interstitial fibrosis and the expression of collagen-1α and transforming growth factor-β1 in the same mouse model [159]. Together, these studies suggest RAAS as a therapeutic target in genetic cardiomyopathies. Despite concerns about the vasodilatory properties of ACE inhibitors and AR blockers in the obstructive form of FHC, the safety and efficacies of candesartan and losartan have been described in studies from patients with nonobstructive FHC [160,161].

Additionally, it has been demonstrated that 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase inhibitors reduce the levels of important molecules in cardiac hypertrophic signaling. Recently, Patel *et al.* [162] have shown that the HMG-CoA reductase inhibitor simvastatin reduced hypertrophy and fibrosis and improved cardiac function in β-MyHC-R403Q TG rabbits by reducing the levels of activated extracellular signal-regulated kinase (ERK) 1/2. Senthil *et al.* [163] have also demonstrated that another HMG-CoA reductase inhibitor, atorvastatin, had similar effects in preventing cardiac dysfunction and remodeling in the same model by reducing the levels of membrane-bound Ras and phospho-p44/42 mitogen-activated-protein kinase (MAPK). Furthermore, treatment of TnT-R92Q TG mice with the antioxidant N-acetylcysteine reduces markers of oxidative stress, 4-hydroxy-2(E)-nonenal and malondialdehyde, expression levels of the mRNAs for procollagen Col1(α1), Col1(α2) and Col3(α1) and the phosphorylation levels of p44, 42, p38 and c-Jun NH2-terminal kinase [164].

Conclusions

Although our knowledge about the genetic and molecular pathophysiology of familial cardiomyopathies has increased substantially in the last two decades, the molecular steps which connect gene defect to clinical phenotype remain elusive. Current data suggest that an extensive panel of causal mutations in a number of different genes can cause cardiomyopathies, and the severity of the disease phenotype is also influenced by several modifier genes. Despite the broad diversity of causal mutations, the signaling response triggered by defective proteins seems to converge into two main phenotypes: FHC, characterized by enhanced contractility, impaired diastolic function and concentric hypertrophy; and DCM, characterized by impaired force generation or transmission, systolic dysfunction and eccentric hypertrophy.

A variety of structural and functional myocardial abnormalities have been identified in animal models of human cardiomyopathies, including defects in the sarcomere assembly, crossbridge kinetics, myofibrillar ATPase activity, myofilament Ca^{2+}-force relationship, excitation-contraction coupling and energetics. In animal models of cardiomyopathies, the expression of FHC- or DCM-linked mutations often results in altered myofilament sensitivity to Ca^{2+} concomitant with abnormal function and expression of Ca^{2+} handling proteins. In agreement with these observations, recent studies have been successful in rescuing cardiomyopathies in animal models by altering either the myofilament response to Ca^{2+} or the Ca^{2+} fluxes that activate myofilaments. Data from animal models of human cardiomyopathies suggest the possibility of developing new treatments for patients with primary and even secondary cardiomyopathies, which would involve direct interventions in myofilament properties or Ca^{2+} regulation. Further studies and more extensive testing are needed to validate these strategies in an attempt to provide more effective and specific treatments for these diseases.

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List of Abbreviations

SR	sarcoplasmic reticulum
RyR	ryanodine receptor
SERCA	sarcoplasmic reticulum Ca^{2+} ATPase
ATP	adenosine triphosphate
PLB	phospholamban

HCM	hypertrophic cardiomyopathy
FHC	familial hypertrophic cardiomyopathy
DCM	dilated cardiomyopathy
MYH7 or βMyHC	beta-myosin heavy chain
TNNT2 or cTnT	cardiac troponin T
TNNI or cTnI	cardiac troponin I
TNNC1 or cTnC	cardiac troponin C
MYBPC3 or MyBPC	myosin binding protein C
TPM1 or α-Tm	alpha-tropomyosin
MYL2 or RLC	regulatory myosin light chain
MYL3 or ELC	essential myosin light chain
ACTC1 or α-actin	alpha-actin
TTN	titin
LV	left ventricle
ACE	angiotensin converting enzyme
AR	angiotensin II receptor
TG	transgenic
NMR	nuclear magnetic resonance
PCr	phosphocreatine
Pi	inorganic phosphate
HF	heart failure
PKA	protein kinase A
PKC	protein kinase C
PKD	protein kinase D
PLBKO	phospholamban knockout
RAAS	renin angiotensin aldosterone system
HMG-CoA	3-hydroxy-3-methylglutarylcoenzyme A
Tm180	Tm E180G
MAPK	mitogen-activated protein kinase
ERK	Extracellular signal-regulated kinase
LIM	specific zinc-binding protein domain
Gαq	subunit Gαq, from the heterotrimeric Gq protein

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Table1

Disease genes for FHC and DCM.

Gene	*Chromosome location*	Number of described mutations	
		FHC	**DCM**
Thick filament			
MYH7 (β-Myosin heavy chain)	14q12	190[165]	13
MYH6 (α-Myosin heavy chain)	14q12	2	3
MYL3 (Regulatory light chain)	3p21.3-p21.2	4	-
MYL2 (Essential Light chain)	12q23-q24.3	10	-
Thin filament			
TNNT2 (cardiac TnT)	1q32	29	7
TNNI3 (cardiac TnI)	19q13.4	27	6
TNNC1 (cardiac TnC)	3p21.3-p14.3	5[166]	1
TPM1 (α-Tropomyosin)	15q22.1	11	2
ACTC1 (α-Actin)	15q11-q14	7	2
Sarcomere-associated and Z-disc proteins			
MYBPC3 (cardiac MyBP-C)	11p11.2	155	3
TTN (Titin)	2q31	2	7
TCAP (T-cap)	17q12	2	1
CSRP3 (cardiac LIM protein)	11p15.1	7	2
ACTN2 (α-Actinin)	1q42-q43	-	1
OBSCN (Obscurin)	1q42.13	2[167]	-
LDB3 (Cypher)	10q22.3-q23.2	-	2
DES (Desmin)	2q35	1	1
DSP (Desmoplakin)	6p24	-	3
MYPN (Myopalladin)	10q21.3	-	4[168]
ANKRD1 (Ankyrin repeat domain)	10q23.33	3[169]	5[170]
MYOZ2 (Myozenin-2)	4q26-q27	2[171]	-
Cytoskeleton/sarcolemma			
CAV3 (Caveolin-3)	3p25	1	-
MVCL (Metavinculin)	10q22.1-q23	-	2
DMD (Dystrophin)	Xp21.2	-	17
SGCD (sarcoglycan delta)	5q33-q34	-	1
Others			
COX15 (Cytochrome c oxidase)	10q24	2	-
LMNA (Lamin A/C)	1q21.2-q21.3	-	39
CTF1 (Cardiotrophin)	16p11.2-p11.1	-	1
TAZ (Tafazzin)	Xq28	-	4
JPH2 (Junctophilin-2)	20q13.12	3[172]	-
PLN (Phospholamban)	6q22.1	-	2
ABCC9 (K_{ATP} channel)	12p12.1	-	2
SCN5A (cardiac Na channel)	3p21	-	3

Gene	*Chromosome location*	Number of described mutations	
		FHC	**DCM**
CRYAB (Crystallin αB)	11q22.3-q23.1	-	2
PRKAG2 (γ2 subunit AMPK)	7q36.1	5	-
TOTAL		470	136

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