Chapter 5

Inherited Cardiomyopathies: From Genotype to Phenotype

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Abstract

The heart undergoes extensive morphological, metabolic, and energetic remodeling in response to inherited, or familial, hypertrophic cardiomyopathies (FHC). Myocyte contractile perturbations downstream of Ca^{2+}, the so-called sarcomere-controlled mechanisms, may represent the earliest indicators of this remodeling. We can now state that the *dynamics* of cardiac contraction and relaxation during the progression of FHC are governed by downstream mechanisms, particularly the *kinetics* and *energetics* of actin and myosin interaction to drive the trajectory of pathological cardiac remodeling. This notion is unambiguously supported by elegant studies above linking inheritable FHC-causing mutations to cardiomyopathies, known to disturb contractile function and alter the energy landscape of the heart. Although studies examining the biophysical properties of cardiac myocytes with FHC-causing mutations have yielded a cellular and molecular understanding of myofilament function, this knowledge has had limited translational success. This is driven by a critical failure in elucidating an integrated and sequential link among the changing energy landscape, myofilament function, and initiated signaling pathways in response to FHC. Similarly, there continues to be a major gap in understanding the cellular and molecular mechanisms contributing to sex differences in FHC development and progression. The primary reason for this gap is a lack of a "unifying" or "central" hypothesis that integrates signaling cascades, energetics, sex and FHC.

Keywords: hypertrophic cardiomyopathy, sex differences, contractility, sarcomere, mutations

1. Introduction to inherited cardiomyopathies

Cardiomyopathies are a major underlying cause of heart failure (HF) and often have a significant heritable component. Although the origin of the inherited trigger can be traced to a single mutation in a sarcomeric gene, the development and progression of a cardiomyopathic phenotype depends on a complex interaction between initiated cellular signaling pathways, environmental stressors, and individual genotype (including sex/gender). Inherited, or familial, cardiomyopathy (FHC) is a disease of the cardiac sarcomere and can be classified as either a hypertrophic (HCM), dilated (DCM), or restrictive (RCM) cardiomyopathy. Inherited cardiomyopathies, FHC, are relatively common in the general population with a penetrance of 1:500 [1].

Although subjects with an identified genotype are at risk for sudden cardiac death (SCD) or HF, it is clinically heterogeneous with severe, mild, or no symptoms. For example, subjects with severe left ventricular (LV) hypertrophy defined as LV thickness >30 mm are at increased risk for SCD [2]. However, subjects with inherited hypertrophic cardiomyopathy (HCM) may present with little LV hypertrophy but yet still be at risk for SCD and HF. Unlike subjects with non-inherited LV hypertrophy, HCM patients may present with cardiac dysfunction that is not proportional to LV thickness [3–5].

HCM is highly progressive and often transitions to DCM. As the name suggests, DCM is characterized by a dilated LV hypertrophy accompanied by worsening cardiac function [6]. Although primary DCM is less common than HCM, DCM is the most genetically heterogeneous [6]. This is best illustrated by findings that DCM can be linked to genes that are also linked to HCM mutations further highlighting the complex nature of FHC.

HCM with abnormal LV diastolic filling associated with intracellular or interstitial infiltration and/or fibrosis in the absence of LV dilation has been described as a RCM. The prevalence of primary RCM is not known, but RCM is less common than both HCM and DCM with poorer clinical outcomes [5]. Again, co-existent HCM and RCM in the same family illustrates the importance of elucidating the pathogenesis that is not entirely based on genotype. Our ability to develop novel approaches or therapies for FHC will depend on a clear understanding of the genotype-to-phenotype interrelationship and potentially translate into personalized treatment options. Since the primary genetic defect in HCM, DCM, and RCM impacts the biophysics of the cardiac sarcomere, we discuss the genotype-phenotype relationship for inherited cardiomyopathies focusing on cardiomyocyte kinetics and energetics in a sex dimorphic manner.

2. Genotype to phenotype

Approximately 100 genes are genetically linked to FHC with most gene mutations encoding for sarcomeric proteins including β-myosin heavy chain (β-MYHC), cardiac troponin T (cTnT), cardiac myosin binding protein C (cMyBP-C), α-tropomyosin (α-Tm), cardiac troponin I (cTnI), myosin regulatory light chain (RLC), and titin [6–8]. FHC follows an autosomal dominant pattern of inheritance and rarely arises from de novo mutations; although founder mutations

are less common, some have been traced to a common ancestor in certain populations and countries [7]. The first identified HCM-causing mutation is a missense in the thick filament gene (*MYH7*) encoding β-MyHC [9]. Since the initial discovery, hundreds of *MYH7* mutations have been genetically linked to HCM and account for 30–40% of all inherited FHCs.

Mutations in *MYBPC3*, a thick filament gene encoding cMyBP-C, is genetically linked to 40–50% distinct HCM-causing mutations, making *MYH7* and *MYBPC3* the most common genes underlying FHC-based disease [3, 10]. Despite the predominance of these gene mutations in HCM, *MYH7* mutations typically result in amino acid substitutions whereas *MYBPC3* variants disrupt the reading frame, leading to a truncated cMyBP-C protein and, often, haploinsufficiency [3]. Considering the role of β-MyHC in cardiomyocyte force generation, a missense mutation within a known critical domain of the *MYH7* gene can be more directly linked to a contractile deficit, usually through a gain-of-function and increased energy cost [11–13]. On the other hand, haploinsufficiency leads to loss of protein or accumulation of truncated protein making it difficult to mechanistically link the mutation to a biophysical effect. In general, patients with *MYBPC3* mutations present with less severe or delayed-onset HCM compared to patients with *MYH7* mutations [3, 6, 10]. Further illustrating profound genetic heterogeneity in FHC-causing mutations, *MYH7* and *MYBPC3* mutations have been linked to inherited DCM as well, even when the mutation is within the same functional domain [2, 6].

Less frequent FHC-causing mutations exist in other thick filament proteins as well. A third thick filament gene linked to FHC, although at a much lower prevalence, is *MLC2*, which encodes for myosin regulatory light chain (RLC). RLC associates with β-MYHC to impact the kinetics of actin-myosin interaction and contractile dynamics [14, 15]. Clinical presentation in patients with *MLC2* mutations is similar to other patients with thick filament mutations, including the phenotypic diversity. Mutations in thin filament proteins (cTnT, α-Tm, and cTnI) have been linked to both HCM, DCM, and RCM [2]. Interestingly, mutations in *TNNT2* (cTnT) and *TPM1* (α-Tm) are potentially more dangerous but with a variable penetrance [4]. For example, subjects are characterized as having "mild hypertrophy" and "less fibrosis", with a significant percentage of SCD patients displaying little to no phenotype [4].

From the above summary, it is clear that a clinical phenotype cannot be categorically assigned to a particular FHC genotype. Still, as a disease of the sarcomere, the prevailing assumption is that FHC-causing mutations perturb the biophysics of muscle contraction. As more sophisticated techniques are available to precisely locate the origins of biophysical abnormalities, investigators remain tasked with detailing the interrelationship between genetic aberrations and basic cardiac sarcomere biology.

3. Sarcomere contractile dynamics

Force generation in a sarcomere is produced by cyclic interactions between myosin and actin, process that is energetically driven by ATP hydrolysis. The myosin-actin interaction is regulated by the tropomyosin and troponin complex; calcium (Ca^{2+}) binding to the troponin complex initiates a macromolecular rearrangement on the thin filament and permits myosin

binding to actin [2, 14]. Contractile perturbations downstream of Ca^{2+} cycling, the so-called sarcomere-controlled mechanisms, represent the earliest indicators of HF [16]. We can now state the *dynamics* of cardiac contraction and relaxation during the progression of HF are governed by downstream mechanisms, particularly the *kinetics* and *energetics* of the cross-bridge cycle to drive the trajectory of pathological cardiac remodeling [17]. This notion is unambiguously supported by elegant studies above linking inheritable FHC-causing mutations to cardiomyopathies, known to disturb contractile function and alter the energy landscape of the heart.

The ability to maintain contractile force at a given cytosolic Ca^{2+} concentration (Ca^{2+}-sensitive tension development) provides an index of cardiac contractility and is often used to characterize the impact of FHC-causing mutations on contractile function [18–21]. While Ca^{2+}-sensitive tension development indexes contractility, the rate of cross-bridge (actin-myosin interaction) binding and unbinding can impart knowledge regarding the amount of force that can be extracted from ATP [22].

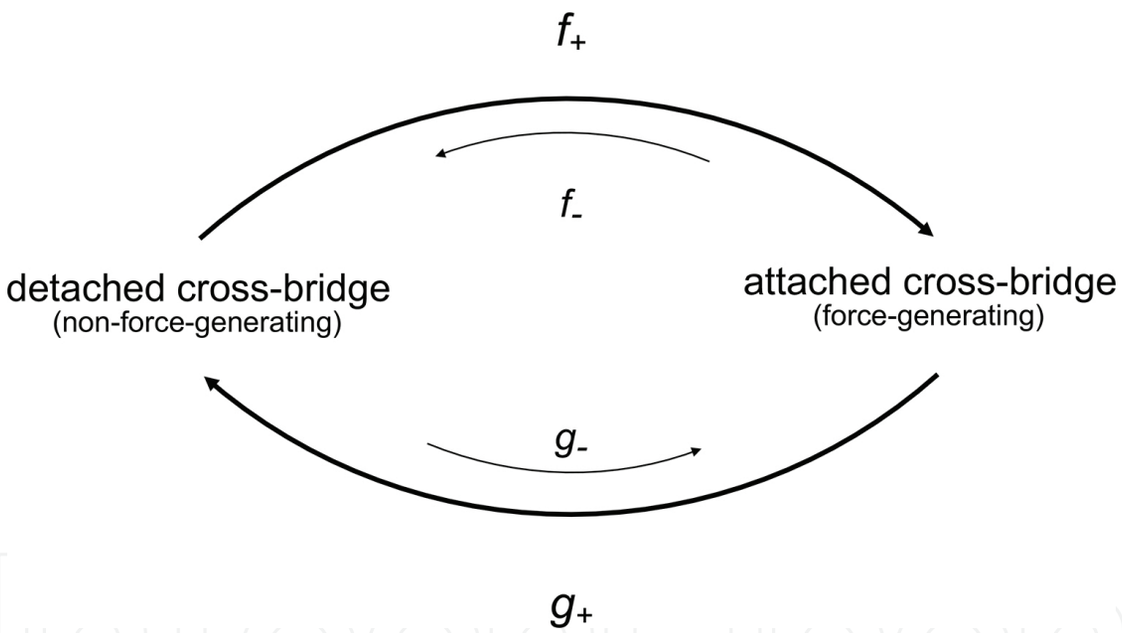

Figure 1. Two-state model of cross-bridge kinetics states that the rate of cross-bridge transition from non-force-generating (detached) to force-generating (attached) is described by f. Similarly, the rate of cross-bridge transition from force generating (attached) to non-force-generating (attached) is described by g. The reverse rate constants, noted as f_- and g_-, are very small. k_{tr} can be defined by $f_+ + g_+$.

The time course of Ca^{2+}-activated tension development following mechanical perturbation (release-restretch protocol) reveals information regarding the kinetics of cycling cross-bridges. Specifically, the rate constant for this time course (k_{tr}) describes the isometric cross-bridge turnover rate, i.e., the sum of the apparent rates of cross-bridges entering and leaving force-generating states using a two-state model system of cross-bridge kinetics [23, 24]. This maneuver can be applied to the muscle at the end of a contraction to measure apparent rate of tension redevelopment (k_{tr}). The recovery of tension toward the isometric steady-state level

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fitted to a single exponential yields the rate constant k_{tr}. We and others define the rate constant k_{tr} according to the two-state model system. Cross-bridge kinetics of the two-state system can be described by rate constants, *f* and *g*, characterizing the making and breaking of cross-bridges [24, 25]. Using this two-state model, k_{tr} can be defined by $f_+ + g_+$ (**Figure 1**).

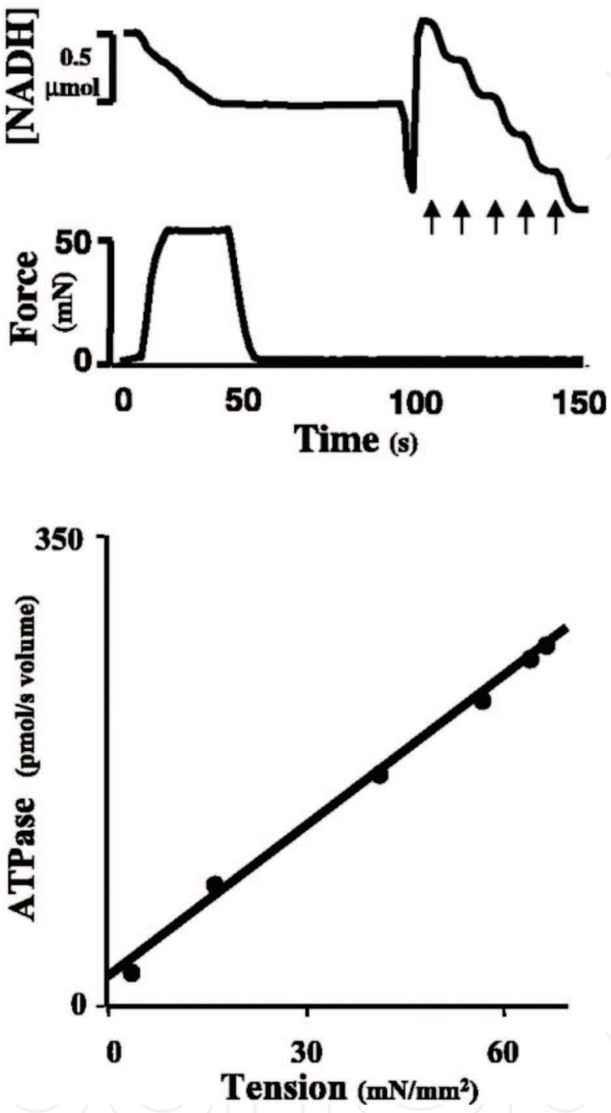

Figure 2. The rate of NADH absorbance is proportional to ATP consumption during isometric steady-state force development (**top panel**). The ATPase rate is plotted against force (**bottom panel**). The slope of this relationship represents the energetic cost (ATPase) of tension generation. Using a two-state model of cross-bridge kinetics, the slope of this line represents the rate of cross-bridge detachment and thus a measure of g. This figure was adapted with permission from Rundell et al. [117].

We have also exploited an apparatus to simultaneously measure force and ATP utilization [26, 27]. Briefly, the bath used for the ATPase assay allows transmission of near-UV light for the measurement of NADH absorbance. The ATPase activity of demembranated cardiac muscle strips or trabecula is measured on-line by means of an enzyme-coupled assay [26, 27]. Formation of ADP by the muscle is stoichiometrically coupled first to the synthesis of pyruvate

and ATP from phosphoenolpyruvate, a reaction that is catalyzed by the enzyme pyruvate kinase, and subsequently to synthesis of lactate, a reaction that is catalyzed by the enzyme lactate dehydrogenase and during which NADH is oxidized to NAD^+. The breakdown of NADH is determined photometrically by measuring the absorbance of 340 nm near-UV light that is projected through the bath just beneath the preparation. Once the steady-state tension is reached, the first time derivative of this signal, which is proportional to the rate of ATP consumption in the assay bath, is determined off-line by linear regression of the sampled data using custom-designed software (**Figure 2**). The rate of ATP consumption, normalized to fiber volume, is plotted against force (**Figure 2**). This slope reflects the tension cost of contraction [26, 28]. Using the two-state model, this value approximates the rate of detachment (g). The combined measurements of k_{tr} and tension cost can give determinations of cross-bridge attachment (f) and detachment (g) [26].

4. ATP shuttling in the cardiac sarcomere

The hypertrophic heart has long been characterized as energy starved [12], and central to this energy remodeling is an alteration in the production, use, and delivery of ATP. Given the physical barriers to rapid diffusion within the myocyte, the cardiomyocyte utilizes key enzymes and a phosphotransferase system to optimize efficient transfer of phosphoryl groups to ADP [29]. Speaking directly to its significance, disturbances in the creatine kinase (CK)/ adenylate kinase (AK) phosphotransfer system are observed early in CVD and are stronger predictors of heart failure mortality than functional status [30]. The molecular underpinnings of the metabolic derangements reside in changes in the mediators of ATP generation, utilization, and delivery. As seen in Eq. (1) creatine kinase (CK) reversibly and rapidly converts ADP and phosphocreatine (PCr) to ATP and creatine (Cr) [31].

$$ADP + PCr \overset{CK}{\rightarrow} ATP + Cr \tag{1}$$

In parallel reactions [Eqs. (1) and (2)], a network of adenylate kinase (AK) enzymes mediates a complementary intracellular phosphotransfer promoting high-energy Pi transfer from ADP to ATP (leaving an increasing AMP pool) via distinct AK isoforms with different cellular localizations [32, 33].

$$ADP + ADP \overset{AK}{\rightarrow} ATP + AMP \tag{2}$$

The diseased heart will preferentially recruit phosphotransferase reactions to keep a constant pool of ATP. As cardiac disease ensues, total Cr and PCr decreases and results in elevated ADP and AMP even if ATP is maintained [11]. Further along the disease process, CK activity is reduced leading to a gradual decrease in cellular ATP [34]. Considering the relatively high rate of ATP synthesis in the heart [12], a gradual decrease in ATP can cause disproportionate

energetic deficiencies [30, 35]. Such changes in energetics limit contractile reserve and the ability to power myocellular ATPases necessary to support contractile function. Given the physical barriers to rapid diffusion within the myocyte, physical association of CK, AK, and other key enzymes in the phosphotransferase system optimizes efficient transfer of phosphoryl groups to ADP [29]. These phosphotransfer microdomains are localized to sarcomeric myofibrils and act as hubs for energetic "sensing" [32, 36]. During acute or chronic ATP supply–demand imbalance, like the one that occurs during cardiac disease, AK amplifies the amount of AMP within these microdomains to preserve ATP levels for contraction [33].

5. Biophysical impact of inherited cardiomyopathies

5.1. Mutations in β-MyHC

The R403Q point mutation in β-MyHC is the first identified mutation leading to HCM with a heritable component [9]. The R403Q mutation resides in the actin-binding domain and *in vitro* analysis of R403Q myosin kinetics yields inconsistent results such as reduced [37] or enhanced [38] actin filament velocity and reduced [39] or enhanced [40] actin-activated ATPase. On the other hand, human myofibril or multicellular R403Q samples consistently show accelerated tension generation and increased ATP hydrolysis rates [20, 41]. In a recent study, we demonstrated that R403Q male fibers develop tension at an increased energy cost of contraction than WT fibers. This is consistent with a previous study that directly measured cross-bridge kinetics in cardiac myofibrils isolated from a patient carrying the R403Q mutation [41]. In addition, R403Q male cardiac trabeculae show an elevated k_{tr} at submaximal tension, again consistent with the kinetics of human R403Q-expressing myofibrils [41]. These observations show that less mechanical energy can be extracted from ATP suggesting an increase in the energy cost of tension generation in R403Q fibers.

Yet, the role that Ca^{2+}-sensitive tension development as an index of cardiac contractility plays in the progression of HCM is unclear. Studies consistently show that Ca^{2+}-sensitive tension development of cardiac fibers is not different between young (6–20 weeks) WT and R403Q hearts [42–44] even though previous studies show that intact hearts from male mice expressing the R403Q mutation show greater contractility compared to controls [45, 46]. We previously showed that cardiac trabeculae from 10-month-old male R403Q trabeculae were not different from wild-type fibers [21]. At the very least, the presence of the R403Q mutation alters myofilament function and ATP utilization at the level of the sarcomere.

5.2. Mutations in cMyBP-C

Historically, cMyBP-C has been viewed as a modifier of contraction through its direct interaction with myosin. In fact, genetic deletion of cMyBP-C in a murine model results in reduced systolic and diastolic parameters, reduced tension at submaximal activation, and cardiac hypertrophy [47]. Representative of a gain-of-function, myocytes lacking cMyBP-C generate more power and display increased rates of force redevelopment at the submaximal activation [48]. Interestingly, permeabilized myocytes taken from humans harboring *MYBPC3* mutations

with evidence of reduced protein expression by haploinsufficiency show reduced maximal force without a change in the kinetics of actin-myosin cycling [49].

In contrast, human samples heterozygous for a missense mutation in *MYBPC3* are more sensitive to activating Ca^{2+}, which may be representative of enhanced contractility [50]. Again, the genetic heterogeneity of *MYBPC3* mutations mirrors the specific impact of each mutation on sarcomeric function. For example, loss of cMyBP-C protein as a result of *MYBPC3* mutations does not necessarily result in "loss-of-function". More recent studies have confirmed that cMyBP-C also interacts with actin to potentially tuning thin filament activation directly while simultaneously maintaining a functional interaction with myosin [51]. The implication for cMyBP-C is a complex regulatory role that imparts novel structural and functional mechanisms during the contractile cycle.

5.3. Mutations in RLC

The myosin regulatory light chain (RLC), also called as the ventricular light chain, is encoded by the *MLC2* gene. The RLC protein forms a non-covalent association with myosin and contains an EF-hand motif implicating functional regulation of myosin and subsequent tension generation [52]. Within this EF-hand "hot spot", the first FHC-causing mutations in the *MLC2* gene were identified, confirming an integral role for the RLC in contractile function [53]. To study the biophysical impact of *MLC2* mutations, mice expressing an E22K RLC transgene were engineered [15, 18]. Hearts from E22K mice display midventricular obstruction due to papillary and septal hypertrophy similar to human counterparts. However, mechanical properties of E22K sarcomeres remain unresolved.

Initial studies using glycerinated cardiac muscle fibers illustrate an increase in Ca^{2+}-sensitivity and Ca^{2+}-activated ATPase of myofibrillar samples from E22K transgenic mice than WT littermate and non-transgenic counterparts [15]. Follow-up studies by the same group report no impact of the E22K RLC mutation on Ca^{2+}-sensitivity of tension and a decrease in maximal ATPase [18]. Inconsistencies in biophysical results from E22K hearts are not surprising, considering the crucial role of RLC phosphorylation in the regulation of myosin mechanics [52, 54]. It is now appreciated that mutations in the *MLC2* gene may impact phosphorylation-dependent changes in force production [54].

5.4. Mutations in cTnT

Along the thin filament, a contractile unit consists of a repeating complex of 7 actin monomers, 1 troponin complex, and 1 α-Tm coil-coil dimer [2]. The heterotrimeric cardiac troponin complex is comprised of cTnI, cTnT, and the Ca^{2+}-binding troponin subunit, cardiac troponin C (cTnC). Myofilament activation hinges on complex molecular interactions between thin filament proteins where Ca^{2+}-binding to cTnC activates the myofilament, changes the position of α-Tm, and removes allosteric inhibition, allowing actin-myosin interaction. Transmission through the contractile unit following Ca^{2+} binding is exquisitely controlled by precise movements of multiple proteins, perhaps predicting a potentially more dangerous phenotype and variable penetrance in patients with FHC mutations residing in thin filament proteins [4].

Missense or splice-site mutations in the *TNNT2* gene result in point mutations or truncated variants resulting in FHC [2, 19]. Unlike *MYBPC3* mutations, truncated cTnT proteins are incorporated into the sarcomere, and early studies in transgenic mice expressing a truncated cTnT modeled after the human condition indicate mild to no cardiac hypertrophy, significant diastolic dysfunction and die more frequently with an increasing allelic expression similar to the human phenotype [55]. Interestingly, cardiac fibers expressing a similar truncated cTnT develop less force at maximum activation [56]. The truncated form of cTnT presumably disrupts the cTnT-cTnI-α-Tm binding domain, but the exact molecular mechanism that leads to the observed phenotype remains unresolved.

Early mapping of cTnT-related FHC alleles intimated the significance of another critical region within the cTnT-α-Tm binding domain [19]. Several FHC-causing substitutions have been identified at residue 92, including R92Q, R92L, and R92W [2]. Hearts taken from mice expressing R92 point mutations are typically smaller, hypercontractile with severe diastolic dysfunction, again, similar to findings in patients with FHC [2, 19, 57]. On the other hand, biophysical studies are inconsistent and depend on experimental approach and the level of molecular resolution. Still, key characteristics can be attributed to R92 mutations, including increased force and actin-myosin cycling at lower Ca^{2+} and less efficient use of ATP to generate force [57–59]. Nevertheless, these biophysical data do not fully explain the phenotypic heterogeneity of cTnT-related FHC arising from genotype-similar patients. Interestingly, a recent work demonstrates significant interplay between cTnT R92 mutations and MyHC isoform [57]. The suggestion from these recent data is that multiple levels of myofilament regulation exist and that specific cTnT FHC mutations cannot be used as surrogates for mutations comprising the functional domain. Clearly, FHC disease progression is a complex integration of myofilament function, cross-bridge kinetics, and cellular signaling, all of which can be modified by environmental and genetic factors such as sex/gender.

6. Sex disparities in FHC

Being male predisposes carriers of FHC-causing mutations to pathological cardiac remodeling [60]. In females, the sharp rise in FHC morbidity and mortality closely aligns with the pre- to post-menopausal transition [60–64]. Despite the longstanding knowledge that pre-menopausal women are protected from developing FHC, our fundamental understanding of the shift in FHC risk with menopause remains inadequate and impedes our ability to develop sex-specific therapeutic strategies to combat FHC and its complications.

The loss of estrogen during menopause positions 17β-estradiol (E2), the predominant naturally occurring estrogen, to play a unique role in cardioprotection. E2 signaling through classical estrogen receptors (ER), ERα and ERβ, and a third, membrane bound and G-protein coupled estrogen receptor (GPER) is initiated by environmental, genetic, and non-genetic cues to impact gene expression and cellular signaling [65–67]. E2-dependent signaling is complex and multiple molecular, and cellular mechanisms have been suggested to underlie protection against CVD [65, 66]. As part of these investigations, studies that utilized gonadectomized

rodents subjected to different cardiac pathological stimuli typically demonstrate a benefit of estrogen replacement [68–72]. Unfortunately, the prospective Women's Health Initiative (WHI) and Heart and Estrogen/Progestin Replacement Study (HERS I and II) studies show an increased CVD and stroke risk with estrogen replacement in menopausal women [73, 74].

Our group has spent the past 15 years studying a murine model of HCM, which expresses an autosomal-dominant R403Q mutation in α-myosin heavy chain and exemplifies this sex dimorphism such that R403Q male mice develop progressive left-ventricular dilation, impaired cardiac function, and a number of pathologic indicators well before R403Q female mice [21, 65, 75–80]. The trajectory of HCM due the R403Q FHC model differs between the sexes in an age-dependent manner. As illustrated in **Figure 3**, adolescent (2 month old) males display cardiac hypertrophy whereas females do not; males progress to a worsening phenotype characterized by ventricular dilation by 8 month of age while females maintain ventricular morphometry with mild hypertrophy [27, 75].

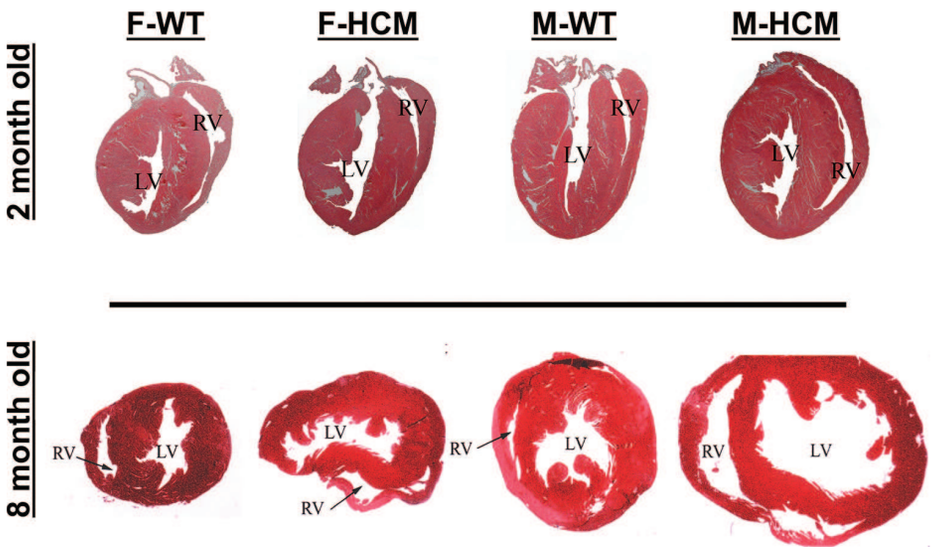

Figure 3. Cardiac morphometry of R403Q female and male mice. **Top panel**: Representative H&E stained longitudinal heart sections from R403Q female and male mice at 2 months of age. **Bottom panel**: Representative H&E stained short axis heart sections from R403Q female and male mice at 8 months of age. This figure was partly adapted with permission from [27].

The biophysical properties of cardiac sarcomeres expressing the R403Q mutation, as for cTnT mutations, depend on the experimental approach. In vitro analysis of R403Q myosin kinetics yields inconsistent results such as reduced [37] or enhanced [38] actin filament velocity and reduced [39] or enhanced [40] actin-activated ATPase. On the other hand, human myofibril or multicellular R403Q samples consistently show accelerated tension generation and increased ATP hydrolysis rates [20, 41]. We and others have reported both age- and sex-dependent effects on Ca^{2+}-sensitivity of tension and the rates of actin and myosin interaction, or k_{tr}. [21, 42, 43]. We further demonstrated that R403Q males show increased cycling (entering and exiting) of cross-bridges at a given force. Furthermore, we find a strong interaction between sex and the R403Q mutation with regard to tension cost. Coupled with measures of k_{tr}, this indicates higher

"off" rate and more inefficient use of ATP at a given force in R403Q males with the opposite effect in R403Q females [27].

What is clear from these studies on the biophysics of the R403Q FHC mutation is that male and female myofilament function is perturbed and potentially under energy stress presumably initiated by the FHC mutation. The underlying mechanisms dictating very distinct disease trajectories in males and females are not completely elucidated by these studies necessitating alternative approaches.

6.1. Role of estrogen in FHC

Sex differences are primarily determined by hormonal status. When considering the effect of sex hormones on the progression of FHC, it is imperative to place a special focus on 17β-estradiol (E2) signaling. Estradiol is the main circulating sex hormone in pre-menopausal women. It is mainly synthesized in ovarian follicles, and to a smaller extent in adipose tissue, liver, breast, and neural tissues [81]. It regulates a number of physiological processes including metabolism, cell growth and proliferation, reproduction, and development [82]. It is generally agreed that estrogen plays a protective role in the myocardium and most of the cardio-protective effects have been attributed to E2. For the rest of this discussion, when we mention estrogen (E2), we are referring specifically to 17β-estradiol.

6.2. Estrogen signaling pathways

Estrogen exerts its physiological effects through interaction with intracellular estrogen receptors (ERs). The first described estrogen receptors, also known as classical estrogen receptors, are ERα and ERβ. They are members of the nuclear hormone receptor family (NHR), contain a DNA-binding domain, and share a high degree of homology [83]. In addition, another estrogen receptor has been recently described. GPER1, a membrane-bound G-protein coupled estrogen receptor, formerly known as GPR30, is a seven trans-membrane domain protein that mediates some of the non-transcriptional activity of estrogen [84].

Estrogen signaling pathways fall into two main categories: genomic (also known as classical) and non-genomic signaling. During genomic signaling, classical estrogen receptors act as transcription factors. After binding to estrogen, they undergo a conformational change that leads to the formation of homodimers (ERα/ERα and ERβ/ERβ) or heterodimers (ERα/ERβ). Receptor-dimers then bind to estrogen response elements (ERE), located near promoter regions of genes, and regulate gene transcription [83]. Estrogen signaling can also be initiated at the non-transcriptional level. Estrogen receptors interact with intracellular proteins triggering signal transduction cascades, often mediated by chain-reaction phosphorylation events. The vast array of estrogen non-transcriptional signaling pathways is mediated by ERα, ERβ, and GPER1 [84, 85].

6.3. Molecular mechanisms of estrogen-mediated cardio-protection

It is most widely accepted that the overall effect of cardiac estrogen signaling has a beneficial outcome on cardiac health. Not only does estrogen mediate cardioprotection, but it is also

involved in the regulation of physiological processes in the heart. ERα expression, for example, is required to maintain physiological glucose uptake and proper mitochondrial function in the murine heart [86, 87]. Acute E2 injections enhance cardiovascular reflexes and autonomic tone in ovariectomy (OVX) mice [88]. Estrogen receptors interact with AMP-activated protein kinase in neonatal rat cardiomyocytes (NRCM), and potentially mediate its activity [89].

More importantly, there is increasing evidence suggesting that estrogen attenuates the progression of cardiac hypertrophy and prevents HF [70, 90]. The molecular mechanisms behind estrogen effects on cardiomyocyte survival are still under study. However, there are many pieces of evidence pointing to specific molecular pathways bridging estrogen signaling and increased tolerance to hypertrophic stimuli such as those arise from FHC. Some of these findings are explored below.

Estrogen has been shown to reverse agonist-induced cardiomyocyte hypertrophy. In NRCM, E2-treatment counteracts angiotensin II (Ang II)-induced increase in cell surface area, protein synthesis, skeletal muscle actin expression, nuclear translocation, and transcriptional activity of the hypertrophic transcription factor NFAT [85]. An E2-dependent increase in SIRT1 expression levels and AMPK activity protects the cardiomyocyte from Ang II-induced injury [91]. E2 reduces cardiomyocyte apoptosis *in vivo* and *in vitro* through the activation of PI3K/Akt signaling [92]. E2 treatment of OVX mice hearts and NRCM inhibits calcineurin activity and increases its degradation [93, 94]. E2 also limits undesirable extracellular matrix (ECM) remodeling through the modulation of ECM protein expression [95]. The majority of the effects discussed above are mediated by signaling through the classical estrogen receptors.

Limited mechanistic insights are available on E2 cardiac signaling mediated through ERα. Selective ERα agonism attenuates cardiac hypertrophy, increasing cardiac output, left ventricular stroke volume, and cardiac α-MyHC expression [96–98]. Signaling through ERβ has been studied in more depth and has been shown to counteract the development of cardiac hypertrophy by reducing the expression of hypertrophic markers, attenuating fibrosis, apoptosis and inflammation [99–101]. ERβ regulates a network of miRNAs, modulates p38 and ERK signaling, and affects calcineurin expression [102, 103]. In fibroblasts, ERβ blocks TGFβ1 synthesis that signals for the production of fibronectin, vimetin, collagens I and III [104].

At least three different mechanisms by which ERβ modulates hypertrophic gene expression in cardiomyocytes have been described. First, E2 signaling through ERβ induces PI3K activation that upregulates MCIP1 transcription [94]. MCIP1 blocks the Ang II-induced increase in calcineurin activity, preventing NFAT translocation to the nucleus and inhibiting the transcription of hypertrophic genes [105]. Second, ERβ signaling can reverse Ang II-induced inhibitory phosphorylation of glycogen synthase kinase-3β (GSK3B) by Akt. This prevents GATA4 transcription factor activation and also leads to decrease in hypertrophic mRNA expression [106]. The third mechanism involves regulation of histone deacetylases (HDAC). ERβ suppress the production and activation of the pro-hypertrophic HDAC2, while promoting the retention of anti-hypertrophic HDAC in the nucleus to inhibit hypertrophic gene expression [106].

Taking into account the complex and multifaceted nature of cardiac estrogen signaling, it is critical to inquire whether the cardioprotective effect of E2 signaling is sex dependent. In the context of cardiac hypertrophy, females show a better response to E2 than males [100], but that does not necessarily mean that E2 signaling is not beneficial for the male heart. It has been shown that E2 treatment of male rats subjected to chronic volume overload attenuates ventricular remodeling and disease progression [107]. It also improves survival in male mice with TNFα overexpression-induced cardiomyopathy [108]. At the cellular level, E2 stimulation of c-kit-expressing cardiac progenitor cells confers cardioprotection against cardiac injury. When co-cultured, ERα stimulation of c-kit + cells enhances the survival of post-infarct male myocytes [109].

In summary, estrogen signaling plays an important role in preventing cardiac remodeling that occurs during hypertrophy and subsequent heart failure. The exact extent to which the different estrogen-targeted pathways contribute to that is still under study. Better understanding of the mechanisms behind estrogen cardioprotection will help to fully understand the sex differences behind the development of FHC, and therefore lead to better and more specialized therapeutic options.

6.4. Menopause models of estrogen depletion

One obstacle that has stalled translational progression of studies into menopausal hypersensitivity to FHC is the lack of appropriate rodent models mirroring progressive ovarian failure, i.e., one that moves from perimenopause into menopause, similar to humans. Most studies have used the surgical removal of ovaries (ovariectomy) as a model of menopause, yet only 10% of women enter menopause surgically. Our studies have utilized an ovary-intact mouse model of menopause, using the chemical 4-vinylcyclohexene diepoxide (VCD) [110]. Repeated short term daily dosing with VCD selectively targets primordial follicles of the ovaries, accelerating the natural process of follicular atresia, and inducing gradual ovarian failure. This model preserves the important "perimenopause" transitional period and androgen-secreting capacity of residual ovarian tissue, analogous to menopausal women [111, 112]. Preserving endogenous androgens in estrogen-deplete females is particularly critical when studying sex differences in FHC [113]. Although androgen levels drop during menopause, the loss of estrogen in menopause elevates the androgen to estrogen ratio and represents an independent risk factor for FHC [114, 115]. We have used this model to demonstrate that during perimenopause, females were protected from hypertension and adverse cardiac remodeling. However after menopause, hypertension and pathological remodeling, indicative of worse clinical outcomes, is a hallmark of this increase in FHC susceptibility during menopause [116]. Importantly, the worsening phenotype in menopausal females is prevented by estrogen.

7. Conclusions

The assertion that FHC is a complex disease is underscored by the difficulty in attributing a single cause to the disease such as aberrant biophysical function of the myofilament. What is

evident from studies of FHC is that although the primary defect may reside in the sarcomere, the development of an HCM, DCM, or RCM phenotype depends on the interaction of the initiated signaling pathways, environmental stressors, and individual genotype (including sex/gender). For example, pathways downstream of Ca^{2+} activation such as Ca^{2+}-sensitivity or actin-myosin cycling kinetics represent functional parameter that is the summation of multiple signals.

Despite an increasing appreciation of sex dimorphisms in the pathophysiology of FHC, many inconsistencies plague the cellular and molecular mechanisms underlying these sex differences. Taken together, there is a clear necessity in elucidating the cellular and molecular actions of estrogen and how this relates to the sex dimorphisms in FHC. Finally, although murine models of FHC do not exactly mimic the human *genotype*, they have proven as useful tools to elucidate the mechanisms underlying the FHC *phenotype*.

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