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Familial Hypertrophic Cardiomyopathy-Related Troponin Mutations and Sudden Cardiac Death

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1. Introduction

Hypertrophic cardiomyopathy (HCM) is a common structural anomaly of the myocardium that is unexplained by an underlying condition such as hypertension. The main findings in HCM are varying degrees of ventricular and/or septal hypertrophy, myocyte disarray and increased myocardial fibrosis (Maron et al., 1995). There is significant variation in the clinical manifestation among patients, from asymptomatic, to mild dyspnea upon exertion, to substantive heart failure. While many individuals will present with clinical symptoms, including a cardiac murmur related to outflow tract obstruction, in some families, diagnosis is not established until the sudden death of, or incidental finding of hypertrophy within, a family member. Transthoracic echocardiography has traditionally been the clinician's primary tool for determination of asymmetric hypertrophy of the left interventricular septum, with or without left ventricular outflow tract obstruction. Given the heterogeneity in severity of disease and penetrance within HCM-affected families, it is important to rule out other secondary causes of hypertrophy, such as hypertension or aortic stenosis. Diagnosis can be difficult, especially in elite athletes who may present with physiological left ventricular hypertrophy (Maron, 2009). Clinically identifiable HCM has a prevalence of 1:500 in young adults in the general population, making it the most common genetic cardiovascular disease in many countries (Maron et al., 1995).

Although familial hypertrophic cardiomyopathy (FHC) was first described clinically more than half a century ago (Teare, 1958), it was only about 20 years ago that the underlying molecular causes of FHC began to be established, with the finding of a mutation in the beta-myosin heavy chain (*MYH7*) gene (Geisterfer-Lowrance et al., 1990). Since this seminal discovery, there have been more than 900 different mutations identified in over 20 FHC candidate genes (Tester & Ackerman, 2009). Historically, attempts to establish the link between genotype and phenotype were based on studying FHC cohorts with severe, well established disease with cardiac remodelling and in some patients, progression to end-stage cardiac dilatation and failure. It is increasingly apparent that focussing on the end phenotype as a link to genotype is problematic; families are highly heterogeneous in their disease presentation with, in many cases, low penetrance (at least on echocardiography diagnoses) and with novel mutations not seen in other families. There are few large FHC-affected families, leading to linkage analysis difficulties. When a pathogenic FHC mutation is uncovered in the proband, genetic testing of all first degree relatives is highly

recommended. When other family members are genotyped, mutation-positive relatives can be closely monitored for disease progression (Colombo et al., 2008).

Up to 60% of patients with a high index of suspicion for FHC are found to have a genetic mutation in one of the FHC-susceptibility genes. A subset of FHC patients do not have identifiable mutations, perhaps because of reduced screening sensitivity that does not incorporate deep intronic sequencing, identify large insertions or deletions in the known candidate genes or include non-hot spot encoding regions. In addition, some patients may have mutations in as-yet unrecognized candidate genes (Rodriguez et al., 2009). The majority of documented FHC mutations occur as single nucleotide substitutions or “missense” mutations, although nucleotide deletions and insertions have also been identified. Insertions and deletions can potentially truncate the gene product by causing a shift in the reading frame leading to a premature stop codon. Mutations that occur at exon/intron boundaries can cause splice anomalies, leading to abnormal and potentially dysfunctional protein products (Wheeler et al., 2009).

Two prominent hypotheses have been developed to explain how sarcomere protein mutations cause the FHC phenotype: first is the “poison polypeptide” hypothesis, in which a single mutant protein disrupts the function of the entire sarcomere unit in a dominant negative manner (Thierfelder et al., 1994). The mutant protein is translated and incorporated into the sarcomere, where it can impair contraction. The second hypothesis is that sarcomeric protein mutations can lead to haploinsufficiency, in which mutations disrupt one copy of the gene, leaving the wild-type gene copy to produce the protein product in inadequate quantities for a balanced sarcomere unit (Thierfelder et al., 1994). In this situation, there is a 50% reduction in peptide concentration due to disruption in translation or trafficking of the mutant. Inadequate levels of incorporated wild-type protein create an imbalance in thin filament stoichiometry.

2. Sudden cardiac death in FHC

FHC is the most common cause of sudden cardiac death (SCD) in young people, affecting approximately 1-2% of children and adolescents, and up to 1% of young adults in HCM community cohorts (Elliott et al., 2000; Maron, 2002). Although SCD is considered rare in competitive athletes (1 in 200,000), HCM is associated with nearly one third of such occurrences (Maron, 2003). Children have the highest SCD rates of FHC patients suggesting that early onset can result from a more severe phenotype that includes lethal arrhythmias (Maron et al., 1999; Ostman-Smith et al., 2008). The highest mortality rates are seen in children aged 9 to 14 years, averaging 7.2%. The SCD risk peaks in girls at 10 to 11 years of age and occurs in boys at 15 to 16 years of age, leading to some researchers to propose that the surge in androgens that occurs prior to puberty may be associated with rapid disease progression and increased SCD risk (Ostman-Smith et al., 2008). There is a male preponderance for FHC-related SCD, especially among athletes (Maron et al., 1996). FHC patients with 2 or more mutations (Hershberger, 2010; Van Driest et al., 2004), and homozygous mutation patients, have more severe disease phenotypes with higher penetrance and greater incidence of SCD over single mutation patients (Ho et al., 2000; Ingles et al., 2005). Modifier gene polymorphisms such as angiotensin I converting enzyme (ACE) D allele, (Marian et al., 1993) and lifestyle/environmental factors such as diet, exercise, body mass and hypertension may also affect the FHC phenotype. With such complexities in disease manifestation, SCD risk assessment has been problematic. Younger

age at onset, history of syncope with exertion, history of SCD within close relatives, severity of symptoms and degree of ventricular and septal wall thickness have been used in risk stratification algorithms; however, many risk factor studies involved non-genotyped patients with sometimes conflicting or confusing results and frequently with no single risk factor being identified. Prognosis for genotyped patients varies with the gene and in many cases, specific mutations within a gene; however, the mechanisms by which such mutations have an increased propensity for sudden death in some individuals, while in others appear to be relatively benign, are not well understood. The primary prevention risk factors for SCD in FHC include family history of SCD, unexplained recent syncope, runs of non-sustained ventricular tachycardia on ambulatory 24 hour Holter monitor, hypotensive response to exercise and severe left ventricular wall thickness (over 30 mm) (Maron, 2010). With respect to the latter, mild ventricular hypertrophy, however, does not correlate with low SCD risk, especially with thin filament mutations, as discussed later.

3. The role of the troponin complex in cardiac dynamics

The focus of this chapter is on three genes that encode the troponin complex found within the sarcomere; *TNNT2*, encoding cardiac troponin T, *TNNI3*, encoding cardiac troponin I and *TNNC1*, encoding troponin C. These genes encode the cardiac troponin genes that are unique from their skeletal counterparts and have evolved to help regulate excitation-contraction coupling in the heart. The troponin (Tn) proteins are part of a thin filament regulatory unit of the sarcomere. Cardiac troponin C (cTnC) is the Ca²⁺-binding subunit that acts as a cytosolic Ca²⁺ sensor, cardiac troponin I (cTnI) is the inhibitory subunit that inhibits contraction when intracellular Ca²⁺ levels are below activation levels and cardiac troponin T (cTnT) is the subunit responsible for attaching the troponin complex to the thin filament via binding with tropomyosin (Tm) and believed responsible for movement of Tm on the thin filament modulating binding of the myosin head to actin. The subunits are arranged in a 1:1:1 stoichiometric ratio along the thin filament with one Tn:Tm complex bound to every seven actin monomers. Actin monomers are arranged in a double helix oriented in parallel to myosin-containing thick filaments. These protein-protein configurations allow for thin filament activation (Figure 1), which in turn facilitates cross-bridge cycling through the action of myosin binding to actin and the production of force (Gordon et al., 2000).

Takeda and collaborators (Takeda et al., 2003) successfully crystallized the globular core of the Tn complex, which revealed that the complex is highly flexible, an inherent feature crucial to its role in heart muscle contraction. The structure consists of two domains: the regulatory head composed of the N-terminus of TnC (residues 3 - 84) and two α -helices of TnI (denoted as H3 and H4, residues 150 - 188), and the highly conserved IT arm composed of the C-terminus of TnC (residues 93-161), two α -helices of TnI (H1 and H2, residues 42-136) and two α -helices of TnT (H3 and H4, residues 203-271). Although crystallography allowed most of the Tn complex structure to be observed, some regions remain unresolved, including the inhibitory region of TnI, and both the N- and C-terminal regions of TnT. These regions are likely highly flexible, allowing them to bind to other thin filament proteins (i.e. actin) to modulate thin filament activation. The primary role of the regulatory domain is as the "Ca²⁺ sensor", while the rigid IT domain appears to be sensitive to myosin binding during contraction (Sun, Bradmeier & Irving, 2006, as cited in Willott et al., 2010).

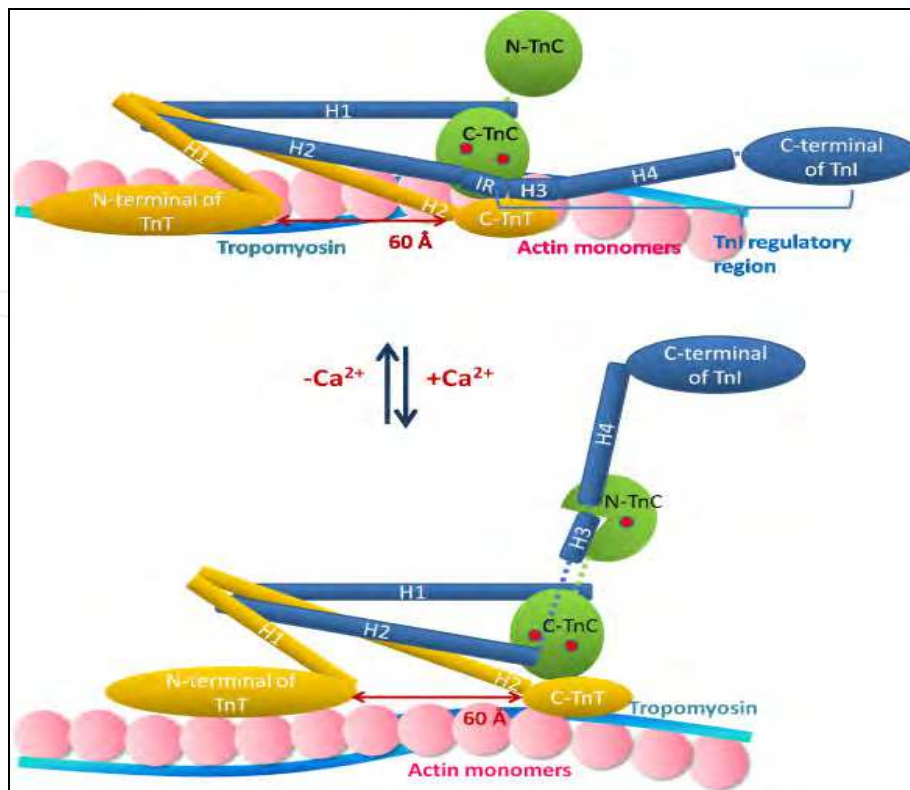


Fig. 1. A schematic representation of cardiac troponin in relation to the thin filament in the absence and presence of Ca²⁺. The inhibitory region of TnI (IR), and the N-terminal and C-terminal domains of TnT are not clearly observed in the crystal structure, likely due to their inherent flexibility (see text). The red dots represent Ca²⁺ ions. The figure is adapted from Takeda, 2003 (Li, 2009).

Most researchers believe that a “3 state model” exists to explain myofibril contraction. Interestingly, it was the study of how various mutations disrupt these interactions that lead to further development and confirmation of the 3 state model (Gordon et al., 2000). During diastole, the ventricles fill with blood to their end-diastolic volumes. The sarcomeres are stretched to longer lengths but without developing significant diastolic pressures. Cross-bridge cycling is physically blocked by the Tm:Tn complex at this stage and is referred to as the “blocked” or “B” state. Recently it has been postulated that perhaps only 50% of the cross-bridges are sterically blocked. The rest may be in a weakly-bound non-force generating state that facilitates the transition of cross-bridge cycling into the systolic state. There are two actin-binding regions on cTnI that play an essential role in diastole. There is an inhibitory region (residues 137-148) and a downstream helix (H3, residues 150-159) that tightly binds to actin, which along with cTnT, anchor Tm into the blocking position (see review by Parmacek & Solaro, 2004).

Calcium initially enters the cell mainly through L-type Ca²⁺ channels and initiates Ca²⁺-induced Ca²⁺ release from the sarcoplasmic reticulum. As cytosolic Ca²⁺ levels rise, the sarcomeres develop tension that increases ventricular isovolumic pressure until the aortic and pulmonary valves open. Blood is expelled from the ventricles by sarcomeres shortening to their end-systolic lengths. At the subcellular level, myofibril activation begins with Ca²⁺ binding to cTnC site II, exposing a hydrophobic region at the N lobe of cTnC and creating a new binding site for cTnI. Cardiac TnI then dissociates from actin and binds

tightly to the hydrophobic region of cTnC, causing a cascade of protein-protein interactions that allows Tm to move closer into the thin filament groove. This stage is referred to as the “closed” or “C” state. This movement exposes myosin binding sites on actin and also appears to alter thin filament structure, allowing more cross-bridges to occur and moving Tm further into the thin filament groove (thus shifting into the “open” or “M” state). Positive feedback may arise from bound cross-bridges causing an increased affinity for Ca^{2+} by cTnC (Pan & Solaro, 1987 as cited in Solaro & Kobayashi, 2011). At basal states of contractility, only 25% of available cTnC regulatory (site II) Ca^{2+} binding sites are occupied due to low cytosolic Ca^{2+} levels, resulting in a substantial cardiac reserve for recruitment of blocked cross-bridges when required.

After the valves close, the sarcomeres are quiescent as the ventricles prepare for refilling. This relaxation phase is highly dependent upon the rate of cytosolic Ca^{2+} removal, the off-loading of Ca^{2+} from cTnC, and the cross-bridges returning to the weakly bound or blocked state. Phosphorylation of the thin filament proteins, in particular the N-terminus of cTnI, plays a crucial role in drawing upon cardiac reserve, cross-bridge cycling rate and hence, relaxation, in a signaling cascade initiated by β -adrenergic stimulation (see review by Tardiff, 2011). These mechanisms are critically important when increased heart rate is required during exercise. Considering that SCD in young FHC patients frequently occurs during exercise (Cha et al., 2007), thin filament mutations may have an inhibitory effect on phosphorylation signalling and cardiac reserve as well as other cross-bridge cycling effects.

4. Mechanisms of Sudden Cardiac Death in FHC troponin mutations

Various mechanisms for SCD due to FHC have been suggested including arrhythmias arising from sinus nodal and atrioventricular nodal conduction abnormalities, and tachycardia due to re-entrant depolarization pathways from myocardial disarray and fibrosis, abnormal Ca^{2+} homeostasis, ventricular diastolic dysfunction or left ventricular outflow tract obstruction (Fatkin & Graham, 2002). With several underlying mechanisms leading to SCD, research is only beginning to define the link between the underlying molecular pathology and arrhythmogenesis in FHC-associated troponin mutations.

One emerging issue is that studying patients with well established disease for the purpose of linking phenotype to genotype has proven extremely difficult. Like all monogenic disorders, there are myriad disease modifiers including genetic, environmental and lifestyle factors that influence disease progression and severity in a manner that is poorly understood. What is also becoming apparent is that ventricular hypertrophy, fibrosis and obstructive disease are likely compensatory FHC features and based on complex signalling cascades arising from pathologies within the sarcomere, as discussed later in this chapter. Perhaps longitudinal studies of patients prior to the onset of structural disease may uncover mutation-specific disease progression that parallels the molecular and biophysical effects observed in *in vitro* experiments, animal models and *in silico* predictions (Tardiff, 2011). There is likely a less complex phenotype in FHC patients in the early disease stages allowing a more discernable link between genotype and phenotype. A study of preclinical FHC patients provides evidence for this hypothesis (Ho et al., 2002). In their study, most FHC cohorts presented with one common phenotype, namely prolonged diastolic relaxation on echocardiography, despite patients having different mutations within different genes. This approach may also benefit treatment outcomes for preclinical FHC cohorts with targeted mutation-specific treatment to attenuate disease progression. It makes sense to treat pre-

symptomatic FHC patients long before gross phenotype becomes established. Diltiazem, a calcium channel blocker, normalized Ca^{2+} regulation and attenuated ventricular hypertrophy in a mouse model (Semsarian et al., 2002) and formed the basis of an ongoing clinical trial, in which preclinical FHC patients receive diltiazem therapy while being monitored for disease progression (<http://clinicaltrials.gov/ct2/show/NCT00319982>).

Recent approaches to identifying the pathophysiology of FHC mutations includes investigation of the dynamic properties of cross-bridge cycling at the molecular level and how Tn mutations disrupt precise molecular movements. Such high resolution investigations commonly incorporate computational approaches to examine protein flexibility and to predict changes in protein mobility caused by mutations, using molecular dynamics simulation programs such as GROMACS (Van Der Spoel et al., 2005) and CHARMM (Brooks et al., 2009). Another approach is Nuclear Magnetic Resonance (NMR) imaging, allowing investigators to compare recombinant wild-type and mutated Tn complexes in different metal-binding states to measure conformational changes (Lassalle, 2010).

4.1 Troponin T mutations

Since the identification of *cTNNT2* in 1993 as the first Tn-based gene associated with FHC (Thierfelder et al., 1993), cTnT mutations have been extensively studied and account for up to 15% of all FHC mutations (Watkins et al., 1995). To date, there are at least 68 cTnT mutations identified associated with FHC (Willott et al., 2010), with a subset that present with a high frequency of SCD and/or ventricular arrhythmia in humans (Table 1). Alternative splicing of exons 4 and 5 of the *cTNNT2* gene in the human heart results in four temporally regulated isoforms: one adult isoform (TnT3) and 3 fetal isoforms (TnT1, TnT2 and TnT4). The variable cTnT N-domain contributes to the Ca^{2+} sensitivity of force development and the presence of fetal isoforms in adult myofilaments has been associated with increased myofilament Ca^{2+} sensitivity and diastolic dysfunction (Gomes et al., 2002 as cited in Gomes et al., 2004). Tn complexes with fetal isoforms TnT1 and TnT2 (containing exon 5) have a reduced inhibition of actomyosin ATPase activity compared with the adult TnT3 isoform which suggests that the TnT isoforms have varying ability to modulate cross-bridge cycling and hence, cardiac contraction (Gomes et al., 2002). These findings are noteworthy in that increased myofilament Ca^{2+} sensitivity and diastolic dysfunction occur with many FHC causing Tn mutations; however, studies investigating the expression of cTnT isoforms in diseased hearts and a possible contributory role in altered contractile performance remain unresolved with no as-yet obvious correlation between fetal isoform TnT4 expression and Ca^{2+} sensitivity in diseased hearts (see review by Parmacek & Solaro, 2004).

The majority of cTnT mutations occur within the two structurally poorly resolved regions with a clustering of mutations within residues 69 to 110. There are three “hot spots” occurring at residues 92, 94 and 110, of which R92L and F110I have been associated with high rates of SCD and/or ventricular arrhythmia (Table 1). Another mutational “hotspot” occurs at residues 160 to 163, which is found within a highly charged and a highly conserved sequence from 157 to 166. This region is believed to be a flexible linker between H1 and H2 and whose structure has so far eluded resolution. Closer to the C terminus is a scattering of mutations associated with dilated cardiomyopathy (DCM) as well as several FHC mutations. It is believed that residues in this particular region affect Ca^{2+} sensitivity via allosteric interactions with the cTnC C domain, although actual evidence is lacking (Tardiff, 2011).

Troponin subunit	Mutation(s)	SCD or Ventricular Arrhythmia	References
cTnT	I79N, F87L, R92L, R92W, R94L, A104V, ΔE160*, S179F, Intron 16G1→A [‡]	SCD	(Gimeno et al., 2009; Knollmann & Potter, 2001; Moolman et al., 1997; Thierfelder et al., 1993; Thierfelder et al., 1994)
	F110I	Ventricular arrhythmia	(Watkins et al., 1995)
cTnI	R145G, A157V, R162Q, ΔK183*, R186Q, S199N	SCD	(Ashrafian et al., 2003; Niimura et al., 2002; Van Driest et al., 2003)
	R141Q, G203R	Ventricular arrhythmia	(Alcalai et al., 2008; Ashrafian et al., 2003)
cTnC	Q122AfsX30 [‡]	SCD	(Chung et al., 2011)

Table 1. Troponin mutations associated with SCD and ventricular arrhythmia. *Δ denotes deletion of the noted residue causing an in-frame mutation; [‡] denotes a splice donor site mutation that removes 28 residues at the C terminus and replaces them with 7 nonsense codons resulting in a truncated cTnT mutant; [‡] denotes a nucleotide duplication (G) at position 363, causing a frame-shift substitution on residue 122 (Q122A) and a premature stop codon (X) at residue 30 resulting in a truncated cTnC mutant.

4.1.1 *In vitro* and *in vivo* approaches, animal models and *in silico* predictions

Cardiac TnT mutations are predicted to affect the regulatory role of the Tn-Tm complex on sarcomere activation given that TnT functions to attach the Tn complex to Tm and actin (Tobacman, 1996). *In vitro* studies with different FHC mutations (including cTnT mutants) show faster contraction kinetics and increased Ca²⁺ sensitivity of force generation. Some studies show increased sarcomeric activation at lower Ca²⁺ levels, resulting in myofilament activation and contraction at shorter sarcomere lengths against an increased passive force (Haim et al., 2007; Tardiff et al., 1999). The shorter baseline sarcomere length may be an important factor in why cTnT R92Q transgenic mutant mice have smaller myocytes and negligent or minimal ventricular hypertrophy (Tardiff et al., 1999).

Abnormal Ca²⁺ homeostasis may result from a variety of factors including altered Ca²⁺ availability and altered myofibrillar Ca²⁺ sensitivity. *In vitro* studies of skinned myocardial fibres reconstituted with mutant cTnT mutations (I79N, R92Q, F110I, ΔE160) show increased myofilament Ca²⁺ sensitivity, which researchers postulate is an important mechanism for the high incidence of SCD even with mild or absent hypertrophy and fibrosis (Gomes & Potter, 2004a; Gomes & Potter, 2004b; Knollmann & Potter, 2001). *In silico* studies based on results from *in vivo* transgenic I79N cTnT mouse fibres predict a higher basal contractility, increased rate of force development, delayed relaxation and increased resting tension compared with wild-type fibres (Miller et al., 2001).

In intact transgenic mouse hearts and in isolated voltage-clamped cardiomyocytes, Knollmann et al. produced compelling evidence that ventricular arrhythmias may arise from action potential remodelling related to altered Ca²⁺ regulation in mice carrying the

human cTnT I79N mutation (Knollmann et al., 2003). A more recent study elegantly demonstrated that the degree of myofilament sensitivity may be correlated positively with the risk of developing ventricular tachycardia (Baudenbacher et al., 2008). Given that the transgenic mice had no evidence of hypertrophy, fibrosis or myocyte disarray, this study provided further evidence that altered myofilament function is the underlying pathophysiological mechanism of FHC and may be the causal link of FHC to SCD. Many of the most “deleterious” cTnT mutations are located within the H1 domain of the N-terminus. Previous studies have demonstrated that the N-terminus plays an important role in the inhibition of myofilament activation (reviewed in Tardiff, 2011). It stands to reason that disruption of this inhibitory N-terminal function by mutations in this region may allow Tm movement (and hence, cross-bridge cycling) under conditions of low Ca²⁺, exhibiting an apparent increased Ca²⁺ sensitivity of myofilament activation (Tardiff, 2011).

4.1.2 Human cardiac TnT mutation studies

FHC patients harbouring the I79N cTnT mutation commonly present with minimal or absent hypertrophy on echocardiography and are frequently asymptomatic (i.e. no syncope, dyspnea or chest pain at rest or with exertion), yet have the highest incidence of SCD among young cTnT mutation carriers (Watkins et al., 1995) and is one of the most investigated of all FHC mutations (see review by Gomes et al., 2004). The F87L mutation also presents with mild hypertrophy (less than 16 mm ventricular wall thickness) but with a high incidence of SCD, including sub-adult patients, in a study of one multigenerational family (Gimeno et al., 2009). Of great significance, the youngest mutation carriers were completely asymptomatic. The R94L was also studied within a single family and found to have marked myocyte disarray and frequent SCD in the absence of ventricular hypertrophy (Varnava et al., 1999 as cited in Gomes et al., 2004). FHC patients with the A104V cTnT mutation also have a high incidence of SCD with only moderate left ventricular hypertrophy (Szczesna et al., 2000 as cited in Gomes & Potter, 2004; Gomes et al., 2004). A longitudinal study involving R92W cTnT FHC patients revealed that clinically identifiable hypertrophy did not occur in this cohort until after 35 years of age and yet the highest occurrence of SCD was prior to cardiac remodelling, particularly in young males (Revera et al., 2007, as cited in Revera et al., 2008). Another study by the same group (Revera et al., 2008) reported that phenotype-negative R92W patients had higher basal contractility and delayed relaxation compared to their genotype-negative relatives. Given the mild phenotype of many cTnT mutation patients, there is likely a reporting and referral bias in these patients and are therefore likely under-recognized in FHC clinics where the majority of patients have substantial hypertrophy and outflow obstruction that are relatively easy to diagnose non-invasively by echocardiography (Tardiff, 2011).

A study comparing FHC cTnT mutation patients with other FHC patients who died suddenly revealed that the cTnT mutation patients were younger, had less hypertrophy and fibrosis, but more myocardial disarray than other patients (Varnava, Elliott, Baboonian et al., 2001). Such findings suggest that the pathological mechanism is essentially myocellular as opposed to being related to the sequelae of ventricular hypertrophy and that ventricular wall thickness may not be an appropriate risk factor for SCD in cTnT patients.

4.2 Troponin I mutations

The first report of FHC-causing mutations in the *TNNI3* gene was in 1997 (Kimura et al., 1997), in which 5 missense mutations were discovered that co-segregated with FHC. Since then, approximately 35 mutations have been reported linked to FHC, of which several missense and deletion mutations are associated with SCD and/or ventricular arrhythmia (Table 1). There are 3 genes encoding 3 TnI protein isoforms, of which two are expressed in the human heart on a temporal basis. The slow skeletal TnI (ssTnI from the *TNNI1* gene) is the predominant isoform expressed within the fetal heart. This isoform declines rapidly after birth and is generally replaced by the cardiac isoform (cTnI from the *TNNI3* gene) by approximately 9 months of age in humans (Bhavsar et al., 1991, Hunkeler, Kullman and Murphy, 1991 and Sasse et al., 1993 in Parmacek & Solaro, 2004). The 31 residue N-terminus in cTnI is entirely absent in ssTnI and contains two serine residues at positions 23 and 24, that are substrates for phosphorylation by protein kinase A (PKA). Given that PKA phosphorylation of these serine residues reduces myofilament Ca^{2+} sensitivity and accelerates cross-bridge cycling during high heart rates (see below), it is not surprising that fetal myofilaments have an increased Ca^{2+} sensitivity and reduced length dependence of Ca^{2+} activation (Arteaga et al., 2000, Fentzke et al., 1999 and Wolksa et al., 2001 as cited in Parmacek & Solaro, 2004). However, increased ssTnI expression is not observed in hearts with severe FHC phenotype (Sasse et al., 1993 as cited in Parmacek & Solaro, 2004), suggesting that other factors, such as myocellular modifications due to FHC mutations, are at play.

The clustering of FHC mutations within the highly flexible inhibitory domain and the mobile region of cTnI with its actin-Tm binding site are of extreme interest to researchers. Perhaps this clustering represents a “tolerance” to alterations of the highly mobile regions of the thin filament, providing evidence that Tn mutations frequently modulate, but do not obliterate, thin filament movements. Some researchers have proposed that under sub-maximal cardiac loads, many of the discussed mutations are relatively benign. However, increased cardiac loads can create the arrhythmogenic substrate leading to SCD in a subset of FHC patients, which is in keeping with the observed high frequency of SCD occurring during or following physical activity (Maron, 2003).

PKA phosphorylation of cTnI affects the cross-bridge cycling rate in response to β -adrenergic activation and represents a post-translational mechanism through which mutations can cause adverse effects to cardiac output and response to increased cardiac demands. Phosphorylation of cTnI at S23 and S24 causes a decrease in Ca^{2+} sensitivity of force generation, increase in off-rates of Ca^{2+} from TnC site II, increase in cross-bridge cycling rate and increase in relaxation rate (Metzger & Westfall, 2004 in Tardiff, 2011). Functional studies with the FHC cTnI R145G mutation provide evidence of an interaction between the N-terminus and the inhibitory domain of cTnI, as the expected desensitization after PKA-mediated phosphorylation was not observed with this mutant. Perhaps the loss of a basic residue (arginine to glycine) depresses inhibition in the inhibitory domain and alters electrostatic interactions with the N-terminal of cTnI (Deng, Y. et al., 2001 as cited in Tardiff, 2011).

4.2.1 *In vitro* and *in vivo* approaches

Similar to other FHC mutations, cTnI mutations demonstrate increased myofilament Ca^{2+} sensitivity which is believed to contribute to pathological hypertrophy and SCD (Parmacek

& Solaro, 2004). Elliot et al. (2000) reported that R145G and R162G mutations demonstrated significantly increased Ca^{2+} sensitivity of ATPase regulation (i.e. force generation) and reduced inhibition of actomyosin ATPase activity *in vitro*. Skinned reconstituted rabbit fibres incorporating R145G, R162G and ΔK183 mutants provide further evidence with increased Ca^{2+} sensitivity consistent with myofilament activation at lower Ca^{2+} levels and predicting an impairment in cardiac relaxation (Takahashi-Yanaga et al., 2001). An *in vivo* study with a cTnI R145G transgenic mouse model also confirmed these results (James et al., 2000). Other animal models recapitulate human FHC findings of myocellular dysfunction preceding structural phenotype; young transgenic cTnI G203S mice display abnormal Ca^{2+} cycling with prolonged decay rates of Ca^{2+} transients long before phenotypic expression of hypertrophy, fibrosis and myocyte disarray (Tsoutsman et al., 2006). Transgenic rabbits expressing low protein levels of R145G cTnI displayed apical myocyte disarray, interstitial fibrosis, but with only mild ventricular hypertrophy at later ages (1.5 to 2 years of age) (Sanbe et al., 2005). Rabbit models more closely resemble human cardiac physiology in that myocellular Ca^{2+} handling and alterations in Ca^{2+} flux during heart failure is much more similar to humans than mouse models (Bers, 2002, as cited in Sanbe et al., 2005). Another limitation for mouse models is their heart rate is roughly 10 times faster than humans, which in turn influences the refractory period associated with arrhythmia incidence (Boyett and Jewell, 1978 as cited in Sanbe et al., 2005).

4.2.2 Human cardiac TnI mutation studies

As with cTnT mutations, studies into patients with cTnI mutations are confounded by small family sizes and referral biases. Nonetheless, characterization of the ΔK183 mutation in several families lead to striking discoveries of high penetrance, age-independent SCD and highly variable ventricular remodeling with some patients, particularly in patients over 40 years, progressing to left ventricular dilatation (referred to as “burned out” hypertrophic cardiomyopathy) within single, multigenerational families (Kokado et al., 2000). A landmark study (Mogensen et al., 2004) reported the phenotype with 748 families ranging from severe restrictive cardiomyopathy, biventricular hypertrophy, or apical hypertrophy in some relatives to no disease features in others, complicating treatment options and risk stratification within families and suggesting that other genetic and/or environmental factors play a role in disease manifestation (Parmacek & Solaro, 2004). Unlike cTnT mutations, however, there have been no reported cases of SCD with mild disease presentation (Mogensen et al., 2004). Interestingly, most of the 13 cTnI mutations within this large cohort are found with the relatively narrow range of exons 7 and 8 encompassing the inhibitory and mobile domains of the C terminal domain.

4.3 Troponin C mutations

Cardiac TnC has only recently joined the list of FHC-causing genes and so far, 6 mutations have been identified (Chung et al., 2011; Chung et al., 2011; Hoffmann et al., 2001; Landstrom et al., 2008; Willott et al., 2010). Cardiac TnC is a highly conserved protein found in all striated muscle among vertebrate species. In mammals, there are two paralogs of TnC: the fast skeletal TnC (sTnC) and the cardiac/slow skeletal TnC (cTnC), consisting of N- and C-terminal domains connected by a long central α -helix. Each domain contains a pair of EF-hand (helix-loop-helix) motifs that bind Ca^{2+} (Kretsinger & Nockolds, 1973, as cited in Li, 2009) and are numbered I to IV (Potter & Gergely, 1975; Zot & Potter, 1982 as cited in Li,

2009). Site III and site IV in the C-terminal domain have high Ca^{2+} binding affinity and are generally occupied by Mg^{2+} and Ca^{2+} ions under physiological conditions. Thus, the C-terminal domain almost always adopts a more open conformation, making it a “structural” domain that maintains the integrity of the Tn complex (Potter & Gergely, 1975; Zot & Potter, 1982 as cited in Li, 2009). The N-terminal domain exhibits a lower Ca^{2+} binding affinity of 10^6 M^{-1} (more than one order of magnitude lower affinity than sites III and IV) and is therefore sensitive to changes in cytosolic Ca^{2+} concentration, making it the “regulatory domain” (Potter & Gergely, 1975; Zot & Potter, 1982 as cited in Li, 2009). It was proposed that the N-terminal domain changes from a “closed” state to an “open” state upon Ca^{2+} binding. A reorientation of helices exposes the hydrophobic residues of the central helix, where the inhibitory domain of TnI binds and triggers the overall conformational change of the Tn complex. As cTnC does not have a functional Ca^{2+} binding site I, it tends to have a more closed conformation compared to sTnC even when site II is coordinating Ca^{2+} (Herzberg, Moulton & James, 1986 in Li, 2009).

4.3.1 *In vitro* analyses and human cardiac TnC mutation studies

What makes cTnC mutations unique is that they are dispersed relatively evenly throughout the gene. As with other Tn mutations, investigations into cTnC mutations are confounded by small family sizes and in some cases, are limited to a single patient. Commercial and research laboratories have only recently added cTnC to their molecular genetic testing platforms (and some continue to omit cTnC from their screenings), leading one to propose perhaps some purportedly genotype-negative FHC patients could potentially harbour cTnC mutations.

The first observed FHC related cTnC mutation, L29Q, was discovered in a 59 year old man who presented with dyspnea upon exertion (Hoffmann et al., 2001). An ECG revealed an abnormal QRS complex suggestive of ventricular hypertrophy and confirmed by echocardiography. There has yet to be any follow-up study with this patient who would now be approximately 70 years of age, which limits knowledge of disease progression with this mutation.

Leucine 29 of cTnC is located in the dysfunctional Ca^{2+} binding site I of the N-domain. Although it is not Ca^{2+} binding, it is important in maintaining the structural integrity of the first helix of cTnC (Sia et al., as cited in Li, 2009). It is located at the cTnI binding site (Schmidtman et al., 2005). Replacement of a non-polar leucine with a polar glutamine is predicted to have an impact on overall function of the Tn complex with Tm. However, several studies show that in the absence of phosphorylated cTnI, the L29Q mutation can decrease, increase, or have no effect on Ca^{2+} sensitivity (Baryshnikova et al., 2008; Liang et al., 2008; Schmidtman et al., 2005) leading to scepticism of its status as a pathogenic FHC mutation. For example, *in vitro* assays conducted on L29Q show that the Ca^{2+} sensitivity of ATPase in reconstituted thin filaments is not affected by PKA-dependent phosphorylation of cTnI (Schmidtman et al., 2005). This finding implies that L29Q may decrease the Ca^{2+} sensitivity and disrupt the signal from the phosphorylated cTnI to cTnC. However, this finding contradicts other reports of FHC mutations generally having higher myofilament Ca^{2+} sensitivities (Chang et al., 2005; Gomes & Potter, 2004; Karibe et al., 2001). Recent NMR and ultraviolet/visual spectrum titration studies showed that L29Q essentially has the same Ca^{2+} affinity as that of wild-type cTnC (Baryshnikova et al., 2008) although this technique presents challenges for measuring Ca^{2+} affinity (see below).

Conversely, our research group demonstrated that L29Q significantly increases the Ca^{2+} sensitivity of force generation using single skinned cardiac myocytes, but in a manner that was extremely sarcomere length dependent (Liang et al., 2008). Cardiac TnC F27W was used as a fluorescence reporter to monitor the *in vitro* Ca^{2+} binding and exchange with binding site II of cTnC. Our results showed that L29Q has a significantly increased Ca^{2+} binding affinity compared to the wild-type, and its response to sarcomere length change was significantly reduced. The increased Ca^{2+} sensitivity suggests that L29Q mutants bind Ca^{2+} more tightly and cause Ca^{2+} to dissociate more slowly from cTnC site II. Reduced length dependence of myofilament Ca^{2+} sensitivity likely influences the heart's ability to regulate ventricular output in response to changes in ventricular filling (Liang et al., 2008). Overall, the heart is maintained in systole longer, resulting in diastolic dysfunction (Wen et al., 2008 in Pinto et al., 2009). Changes in Ca^{2+} affinity is hypothesized to disrupt myocellular homeostasis, triggering Ca^{2+} -regulated pathways leading to SCD (Baudenbacher et al., 2008) and/or hypertrophy (Heineke & Molkentin, 2006) as discussed later in this chapter.

Choice of experimental techniques may account for the conflicting results. Compared to studies using single cardiac myocytes, ATPase and *in vitro* motility assays are excellent techniques for defining molecular interactions, but lack the geometric and mechanical constraints from other proteins within the sarcomere (Liang et al., 2008). Additionally, NMR techniques are limited in terms of accurate Ca^{2+} measurement as Ca^{2+} chelators, such as EGTA, cannot be utilized in NMR studies (Liang et al., 2008). The reduced length dependence of Ca^{2+} sensitivity is likely why other researchers, who had no or only marginal sarcomeric length control in their experimental techniques, have observed such variable results. Our experimental technique using single cardiac myocytes held at a constant sarcomere length may be more precise and closer to physiological conditions. Further to this, our work on cTnC and the L29Q mutation precedes the discovery of a human L29Q cTnC FHC patient. Salmonid cTnC has a greater than two-fold Ca^{2+} affinity over mammalian cTnC (Gillis et al., 2003) with four sequence differences between the mammalian and salmonid homologues responsible for the high Ca^{2+} affinity: D2N, V28I, L29Q, and G30D (NIQD). When the mammalian residues were mutated to the salmonid-equivalent, including L29 to Q29, the Ca^{2+} -binding affinities of the mammalian cTnC mutants increased to the level of the salmonid cTnC (Gillis et al., 2005).

Seven years after the initial FHC cTnC report, a large study cohort of 1025 unrelated patients was screened for FHC mutations and four novel cTnC mutations were reported: A8V, C84Y, E134D and D145E (Landstrom et al., 2008). All four patients were symptomatic for FHC, with findings of syncope upon exertion (C84Y) and dyspnea and chest pain in the other three patients. They were all positive for varying degrees of ventricular hypertrophy. All were young or relatively young (17, 22, 37 and 58 years old) when diagnosed, but SCD was not reported in any patients or relatives. Functional analysis of the four variants using skinned porcine papillary fibres revealed increased Ca^{2+} sensitivity of force development for A8V, C84Y and D145E mutations, and A8V and D145E also showed increases in maximal force consistent with other *in vitro* studies of FHC-associated mutants (Landstrom et al., 2008). Actomyosin ATPase activity in reconstituted thin filaments and spectroscopic properties of the four mutants confirmed increased myofilament Ca^{2+} sensitivity, except for E134D which was not significantly different from wild-type (Pinto et al., 2009). Isolated cTnC, Tn complex and thin filament assays, however, did not recapitulate the Ca^{2+} sensitivity findings observed in the reconstituted fibre assays, suggesting that the entire

reconstituted myofilament (that included the S1 myosin head) is required to recreate the increased Ca^{2+} sensitivity changes observed in skinned fibre assays.

This research group also proposed that the D145E mutation influences regulation of contraction by disrupting Ca^{2+} binding to site IV of cTnC and demonstrated that this mutation reduced the cTnC α helicity in the metal-bound state as determined by circular dichroism (Pinto et al., 2009). Another report investigated the effects of IAANS-labeled cTnC mutants on Ca^{2+} off-rate kinetics and concluded that both A8V and D145E mutations had significantly slower off-rate kinetics over the wild-type, suggesting that both mutations alter muscle relaxation properties by reducing ventricular filling time which correlates with the diastolic dysfunction seen in FHC patients (Pinto et al., 2011).

Only one cTnC mutation so far has been directly linked to the SCD of a previously undiagnosed and asymptomatic 19 year old man who had a witnessed collapse while working at his computer (Chung et al., 2011). Autopsy revealed ventricular hypertrophy. Genetic testing of his family revealed a novel cTnC duplication at nucleotide 363, leading to a frameshift mutation at Q122A and causing a premature stop codon at position 30 in the new reading frame (Q122AfsX30) in 4 out of 7 relatives. The primary concern was for his 16 year old genotype-positive, phenotype-negative sister, who can be monitored for disease manifestation (Chung et al., 2011). To date, no functional analysis has been done on this mutation finding. The premature stop codon created by this frameshift mutation is close to the C terminus, leading to speculation that the mutant protein is successfully translated, incorporated into the thin filament and creates adverse effects on Ca^{2+} sensitivity of force production similar to other cTnC mutations. One could also propose that the protein undergoes nonsense-mediated decay, leading to haploinsufficiency of cTnC within the cardiac myocytes. Future investigations will hopefully provide further insight to the pathogenic mechanisms of this newest cTnC mutation finding.

5. Arrhythmogenic mechanisms in FHC

Many studies comparing FHC phenotype with suspected underlying mechanisms are plagued by the lack of genotyping of cardiomyopathy patients, perhaps related to the high cost and time-consuming work of genetic testing. To address this issue, Colombo et al. (Colombo et al., 2008) argue that some genotype-phenotype correlations can provide important information to target DNA analyses in specific FHC candidate genes. Genetic testing may also clarify diagnosis and assist with optimal treatment strategies for more malignant phenotypes. In addition, genetic screening of first-degree relatives can assist in early identification and diagnosis of individuals at greatest risk for developing cardiomyopathy, allowing physicians to focus clinical resources on high-risk family members. Determining the underlying mechanism of SCD resulting from FHC remains elusive, though recent studies have begun to focus on the three cardinal manifestations of FHC separately (i.e. cardiac hypertrophy, myocyte disarray and fibrosis), as researchers are postulating that they may arise from distinct and independent mechanisms (Varnava, Elliott, Baboonian et al., 2001; Varnava, Elliott, Mahon et al., 2001; Wolf et al., 2005). Myocyte hypertrophy is postulated by some to increase arrhythmia vulnerability through intrinsic automaticity changes, as some studies demonstrate that hypertrophied myocytes exhibit pacemaker current up-regulation (re-expression) and action potential prolongation by down-regulation of the potassium transient outward I_{to} current (Sanguinetti, 2002 as cited in Wolf et al., 2005).

Triggered arrhythmias can occur as delayed after-depolarizations (DADs), early after-depolarizations (EADs) or increased automaticity in non-ischemic FHC and are likely related to myocellular Ca^{2+} signalling and transport (Bers, 2008). DADs are commonly believed to be caused by spontaneous Ca^{2+} release from the SR that occurs as a consequence of high SR Ca^{2+} levels. This SR Ca^{2+} release causes a transient inward current (I_{ti}) that can cause a threshold depolarization leading to an action potential. Several studies suggest that the $\text{Na}^+/\text{Ca}^{2+}$ exchanger current (I_{NCX}) is responsible for I_{ti} in human ventricular myocytes (Pogwizd et al., 2001). Further to this, Ter Keurs' research group has been investigating myofilament arrhythmogenic Ca^{2+} release to determine if non-uniform excitation-contraction coupling plays a role in the initiation of extra-systoles that create arrhythmias ((Ter Keurs et al., 2006). Ter Keurs developed a model of non-uniform excitation-contraction using rat trabeculae and exposed a small muscle segment to BDM, a cross-bridge inhibitor (Backx et al., 1995 as cited in Ter Keurs et al., 2006), to recapitulate non-contracting myocardium as found in diseased hearts. Triggered propagating contractions were observed in the border zone of myocardium between non-contractile and contractile tissue when the trabeculae were stimulated to contract. The triggered contractions may be due to a quick release-induced Ca^{2+} dissociation from cTnC site II, leading to a local Ca^{2+} surge that is above the threshold for inducing Ca^{2+} -induced Ca^{2+} release. This mechanism, referred to as "reverse excitation contraction coupling" (RECC), occurs when Ca^{2+} reuptake mechanisms have sufficiently recovered from the previous contraction during diastole (Boyden & ter Keurs, 2001). In FHC, the myocardium may have focal regions of non-uniformity due to structural anomalies, such as fibrosis or myocardial disarray, or perhaps due to electrical remodelling or gene dosage effects from Tn mutations. Increased Ca^{2+} binding to cTnC leading to a high Ca^{2+} buffering capacity may cause a large Ca^{2+} surge during rapid myofilament shortening during RECC. Hence, RECC may be an underlying pathological mechanism of arrhythmogenesis seen in FHC patients and warrants further investigation.

Electrical alternans, in which there is alternating long and short action potential duration (APD), increases the risk of ventricular tachycardia that can degrade to ventricular fibrillation and SCD. The underlying mechanism may be related to Ca^{2+} transient amplitude alternans. At high pacing frequencies, slow ryanodine receptor and/or Ca^{2+} current recovery may result in alternating SR Ca^{2+} release, due to alternating availability of ryanodine receptors (RyR). Prolonged Ca^{2+} transient decay rates, as seen in some transgenic FHC animal models may play a role here (see below). Spatially discordant alternans is thought to be a prerequisite to dangerous arrhythmias, as there is a non-synchronous electrical substrate within the heart (Bers, 2008). Animal models support this hypothesis; increased myofilament Ca^{2+} sensitivity was associated with an arrhythmogenic substrate in transgenic cTnT mice, despite the absence of structural heart disease (Baudenbacher et al., 2008). Addition of a myofilament Ca^{2+} sensitizing agent, EMD, resulted in repolarization alternans at high pacing rates, beat to beat variation in APD, shorter effective refractory periods and increased spatial conduction velocity dispersion in wild-type cat and mouse hearts, paralleling the findings as observed in mutant cTnT I79N transgenic mice. Several mechanisms were proposed for the induction of ventricular tachyarrhythmias: increased Ca^{2+} binding to cTnC resulting in reduced Ca^{2+} transients with slower decay rates responsible for the shorter APD seen in transgenic I79N cTnT mice, and dysfunctional myocardial relaxation as seen in transgenic mice and in human patients also causing APD shortening. However, transgenic mice have differing

ion channel and Ca²⁺-handling protein expression from human hearts (Wetzel & Klitzner, 1996). The high heart rates of mice, roughly 10 times faster than humans, can influence the refractory period associated with the incidence of arrhythmias, as mentioned previously. Additionally, studies of human HCM patients suggested that T-wave alternans (surface ECG recording associated with action potential alternans) may not be a useful SCD prediction tool (Fuchs & Torjman, 2009).

5.1 Clinical findings in FHC patients

It has proven difficult to elucidate the exact mechanisms linking FHC pathology and arrhythmogenicity in humans with limited access to fresh cardiac tissue from FHC patients. Studies currently utilize tissue from myectomy samples and explanted hearts in which there is profound disease phenotype. Thus, explorations of disease progression in pre-clinical patients are normally limited to non-invasive imaging techniques and electrophysiology. In addition, there is inherent patient referral bias for research studies as most FHC patients seen in surgical referral centres already have profound disease manifestation (Tardiff, 2011).

Stored electrograms from implantable cardiac defibrillators indicate that SCD largely results from sustained ventricular tachycardia and/or ventricular fibrillation (B.J. Maron, 2010). One suggested trigger for SCD is sympathetic excitation, given that the initiating rhythm in many cases is sinus tachycardia, which may underlie the high SCD rate in athletes and sub-adult FHC populations (Cha et al., 2007). Several studies of HCM patients wearing Holter monitors identified a higher rate of SCD for younger patients (age 30 and under) with non-sustained ventricular tachycardia (NSVT) detected at least once during the 48 hour monitoring period. While NSVT is usually asymptomatic and frequently occurs during periods of increased parasympathetic (vagal) tone, it is associated with an increased SCD risk, especially in children and young adults (Elliott et al., 2000; Monserrat et al., 2003). However, most SCDs occur in patients without ambulatory ECG episodes of NSVT; clearly other contributory factors leading to risk of SCD are at play. A more recent study identified an increased SCD risk with ventricular arrhythmias triggered by exercise (Gimeno, Tome-Esteban et al., 2009), which is more in keeping with findings of triggered arrhythmias during sinus tachycardia, implicating sympathetic excitation during physical activity (Cha et al., 2007). This is also in keeping with the *in vitro* experimental evidence of a blunted response to β -adrenergic stimulation through phosphorylation of cTnI (i.e. reduced inhibitory response of phosphorylated cTnI on Ca²⁺ sensitivity for cTnC) and the resultant diastolic dysfunction as discussed previously.

Cardiac magnetic resonance (CMR) imaging has allowed clinicians to precisely determine myocyte fibrosis and scarring in non-ischemic FHC, including phenotype-negative FHC patients who have experienced life-threatening arrhythmias (Makhoul et al., 2011; Strijack et al., 2008). Detection of fibrosis by late gadolinium enhancement in CMR is associated with increased propensity for VT on ambulatory ECG monitoring (Adabag et al., 2008) and is being considered as a clinical SCD risk marker (Maron, 2010). Fibrosis and scarring may promote localized zones of slowed conduction resulting in re-entrant arrhythmias (Cha et al., 2007). NMR imaging can detect focal or diffuse regions of fibrosis and hypertrophy morphologies that are missed by traditional echocardiography (M. S. Maron, 2009) and will likely continue to improve in resolution leading to improved risk stratification for FHC patients.

Myocyte disarray is another common feature in FHC patients and a recent study of myectomy samples from a small pediatric HCM cohort suggests that myocyte disarray has a significantly higher correlation with diastolic dysfunction than either hypertrophy or fibrosis (Menon et al., 2009). Extensive myocyte disarray has been linked to SCD in younger FHC patients (Varnava, Elliott, Mahon et al., 2001), especially those with cTnT mutations (Varnava, Elliott, Baboonian et al., 2001) in the absence of, or with minimal hypertrophy.

6. Future considerations

Of interest to investigators is the potential role of Ca^{2+} dysregulation in ER stress pathways. The SR in myocardial cells has long been thought to be the cardiac equivalent to ER with its main role as the intracellular regulator of Ca^{2+} fluxes and hence, excitation-contraction coupling in the heart. Some researchers propose that the SR contains a functional ER “compartment” with physiological roles such as protein synthesis, translocation and integration into membranes, folding and post-translational modifications including glycosylation and Ca^{2+} homeostasis (Mesaeli et al., 2001), although studies are lacking. ER stress occurs in response to environmental or genetic factors causing ER metabolic disturbances, accumulation of misfolded proteins, oxidative stress and/or depletion of ER Ca^{2+} stores. The “unfolded protein response” (UPR) is one mechanism by which the ER attempts to reestablish homeostasis by reducing protein expression, by increasing production of chaperones to handle accumulation of misfolded protein, promoting ER-associated degradation to remove misfolded proteins (Schroder & Kaufman, 2005). This initial response of protein synthesis, suppression and upregulation of ER resident chaperones is designed to resolve the ER stress and enhance survival, but if the ER stress is severe or prolonged, the UPR may stimulate apoptosis (cell death). Do FHC mutations play a role in ER stress pathways, such as through Ca^{2+} dysregulation? Activation of the “fetal gene program” is a response to elevated Ca^{2+} to increase cardiac efficiency in the stressed heart. This response, unfortunately, also commonly results in detrimental cardiac hypertrophy (Eizirik et al., 2008; Wang et al., 2000). Elevated Ca^{2+} activates calcineurin A that dephosphorylates the transcription factor NFAT which translocates to the nucleus to stimulate cardiac remodelling and hypertrophy associated with the fetal gene program (Heineke & Molkentin, 2006). Other factors, such as MEF2, GATA and CamKII are also activated which initiate transcription programs associated with hypertrophy, remodelling and heart failure with increased risk of cardiac death (Molkentin et al., 1998). GATA-4 may play a significant role in FHC pathogenesis due to its ability to stimulate transcription of the cardiac-specific Tn genes (Liang et al., 2001; Molkentin & Olson, 1997; Molkentin et al., 1998). Therefore, ER stress may turn on the fetal gene program in response to pathological insult. MicroRNAs (miRs) likely play a role in these regulatory processes with miRNA coding sequences often located within the newly transcribed genes (Eizirik et al., 2008; Wang et al., 2000). The hypothesis of elevated Ca^{2+} causing transcriptional activation of hypertrophy and perhaps pathological arrhythmia substrates (see below) is provocative and will likely continue to be an area of active investigation.

Research into miRs that play a role in regulation of cardiac function and the recent findings that miR expression is deranged in cardiac disease may help to uncover the pathways to FHC disease progression and arrhythmogenesis. MiRs are short, non-coding RNA sequences that regulate expression of genes involved in orchestrating growth, development, function and stress responses in a spatio-temporal manner. MiRs target specific mRNA

sequences generally to inhibit protein expression, either by degradation of the bound mRNA target or by directly inhibiting translation of the mRNA sequence (Bartel, 2004). To date, there are at least 4 miRs shown to be involved in cardiac development, apoptosis and hypertrophy, namely: miR-1, miR-133, miR-208 and miR-499 (van Rooij et al., 2006). Several important target genes for miRs related to cardiac electrophysiology have been identified, including connexin 43 and inwardly-rectifying potassium channel Kir2.1 (Zhao et al., 2007) and miR-1 expression changes have been associated with arrhythmogenesis due to up-regulation or down-regulation of these gene products (Girmatsion et al., 2009; Yang et al., 2007). Recent studies are beginning to elucidate the link between miRs and SCD due to arrhythmias by identifying the effects of altered expression levels of miRs in the heart on cardiac conduction and excitability (Callis et al., 2009; Matkovich et al., 2010; Zhao et al., 2007). A database has been developed online (<http://www.mir2disease.org/>) for miRs involved in human disease, including FHC.

Sudden Infant Death Syndrome (SIDS) refers to the sudden death of an infant under 1 year of age which remains unexplained after a thorough medicolegal investigation (Willinger et al., 1991). Researchers are now considering inherited cardiac arrhythmia syndromes in its etiology. Recent research has revealed that up to 20% of SIDS cases may be associated with inheritable arrhythmia syndromes, such as long QT syndrome (Klaver et al., 2011). Given that FHC is the most common cause of SCD in young individuals, it stands to reason that some infants may die from SCD attributed to FHC. Further to this, as discussed earlier, some Tn mutants, particularly the cTnT mutants, have negligible or mild hypertrophy that may not be observed grossly during the post mortem exam. To date, only one study has screened SIDS cases for FHC mutations (Brion et al., 2009). Their findings of 14 cases with 7 genetic variants from 4 different FHC genes, including cTnT and cTnI, from 140 SIDS tissues suggests that some SIDS cases may be associated with FHC-causing mutations. The relatively recent emergence of FHC-associated Tn (in particular, cTnC) gene mutations make these candidate genes previously unrecognized and perhaps under-represented factors to be considered in future SIDS investigations. Besides SIDS cases, how many FHC mutation positive cases have gone unrecognized in post mortem investigations following the sudden, unexpected death of children and young adults?

7. Conclusions

Sudden cardiac death affects approximately 1-2% of children and adolescents, and up to 1% of young adults in FHC-affected populations. *In vitro* analysis of single molecule mechanics and reconstituted skinned myocytes have identified intracellular Ca²⁺ dysregulation, altered myofibrillar Ca²⁺ sensitivity and altered energy metabolism as potential mechanisms at the sarcomere and cellular level. Animal models incorporating specific FHC mutations have broadened our understanding of the pathogenesis of FHC, including structural and electrophysiological remodelling associated with the arrhythmogenic substrate. There are, however, caveats to using *in vitro* and animal model analyses, given that some may not necessarily recapitulate the physiological substrate in human FHC patients. Most models, however, share a consistent molecular phenotype, namely increased myofilament Ca²⁺ sensitivity and increased energetic cost of force development, that underlies the complex and heterogeneous phenotype that exists at the human patient level. Other genetic, environmental and biological factors such as age, lifestyle and other health issues are also likely disease-modifying factors. Research into molecular approaches and post-translational

mechanisms associated with FHC, including effects of phosphorylation and a potential role in ER stress mechanisms, will likely continue to elucidate the link between genotype and phenotype.

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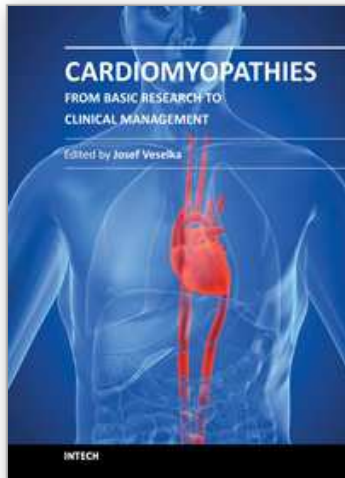
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Cardiomyopathy means "heart (cardio) muscle (myo) disease (pathy)". Currently, cardiomyopathies are defined as myocardial disorders in which the heart muscle is structurally and/or functionally abnormal in the absence of a coronary artery disease, hypertension, valvular heart disease or congenital heart disease sufficient to cause the observed myocardial abnormalities. This book provides a comprehensive, state-of-the-art review of the current knowledge of cardiomyopathies. Instead of following the classic interdisciplinary division, the entire cardiovascular system is presented as a functional unity, and the contributors explore pathophysiological mechanisms from different perspectives, including genetics, molecular biology, electrophysiology, invasive and non-invasive cardiology, imaging methods and surgery. In order to provide a balanced medical view, this book was edited by a clinical cardiologist.

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