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Beyond the Cardiac Myofilament: Hypertrophic Cardiomyopathy-Associated Mutations in Genes that Encode Calcium-Handling Proteins

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Abstract

Traditionally regarded as a genetic disease of the cardiac sarcomere, hypertrophic cardiomyopathy (HCM) is the most common inherited cardiovascular disease and a significant cause of sudden cardiac death. While the most common etiologies of this phenotypically diverse disease lie in a handful of genes encoding critical contractile myofilament proteins, approximately 50% of patients diagnosed with HCM worldwide do not host sarcomeric gene mutations. Recently, mutations in genes encoding calcium-sensitive and calcium-handling proteins have been implicated in the pathogenesis of HCM. Among these are mutations in *TNNC1*-encoded cardiac troponin C, *PLN*-encoded phospholamban, and *JPH2*-encoded junctophilin 2 which have each been associated with HCM in multiple studies. In addition, mutations in *RYR2*-encoded ryanodine receptor 2, *CASQ2*-encoded calsequestrin 2, *CALR3*-encoded calreticulin 3, and *SRI*-encoded sorcin have been associated with HCM, although more studies are required to validate initial findings. While a relatively uncommon cause of HCM, mutations in genes that encode calcium-handling proteins represent an emerging genetic subset of HCM. Furthermore, these naturally occurring disease-associated mutations have provided useful molecular tools for uncovering novel mechanisms of disease pathogenesis, increasing our understanding of basic cardiac physiology, and dissecting important structure-function relationships within these proteins.

Keywords

Calcium; genetics; hypertrophic cardiomyopathy; junctophilin; mutation; phospholamban; troponin

HYPERTROPHIC CARDIOMYOPATHY

Hypertrophic cardiomyopathy (HCM) is a common cardiovascular disease defined clinically as otherwise unexplained thickening of the ventricular walls and/or interventricular septum. First described by Teare as “asymmetrical hypertrophy of the heart” in eight young adults, five decades of intense clinical, translational, and basic science investigation have been

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CONFLICT OF INTEREST

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dedicated to this disease [1]. HCM is the most common inherited cardiovascular disorder affecting approximately 1 in 500 persons. It is a significant cause of youthful sudden cardiac death (SCD) and the most common cause of SCD in athletes [2–4]. Grossly, HCM is characterized by asymmetrical left ventricular (LV) hypertrophy with possible involvement of the right ventricle. At the histological level, HCM is defined by myocyte hypertrophy and myofibrillar disarray with interstitial fibrosis.

HCM demonstrates phenotypic heterogeneity with a spectrum of clinical features. The degree of cardiac hypertrophy is variable between probands as is cardiac fibrosis, myocyte disarray, LV outflow tract obstruction, and sudden death susceptibility. Further, abnormal intramural coronary arteries, systolic anterior movement of the mitral valve, and arrhythmias can be variably present [5]. Presenting symptoms typically include chest pain, dyspnea, palpitations, and syncope; however, SCD may also occur in the absence of major symptoms [6]. Just as clinical presentation is variable, clinical progression is largely unpredictable, with some patients remaining asymptomatic over their lifetime while others present as neonates with profound cardiac hypertrophy [7]. Cardiac arrhythmias can occur such as atrial fibrillation and non-sustained ventricular tachycardia [8]. 5–15% of patients with HCM will experience further pathogenic remodeling ultimately leading to heart failure with LV wall thinning, chamber dilatation, and loss of cardiac contractility [9, 10]. In this way, HCM is not a static disease with consistent clinical findings, but rather one that may or may not include a number of clinical characteristics that can evolve over time.

The variable clinical phenotype of HCM reflects both the diverse genetic basis of the disease and the myriad of factors which can modify disease expression. Indeed, HCM has been appreciated as principally an autosomal dominant disease with variable expressivity and incomplete penetrance. Hundreds of mutations found in dozens of genes encoding various sarcomeric/myofilament, Z-disc, and calcium (Ca^{2+})-handling proteins have been associated with this disease (Table 1).

HCM DUE TO CARDIAC MYOFILAMENT/SARCOMERE MUTATIONS

Myofilament/Sarcomeric-HCM

HCM is the first myocardial disorder in which the genetic basis of disease pathogenesis has been elucidated. The discovery of a genetic locus responsible for familial HCM was first identified over 20 years ago utilizing linkage analysis of a large, multi-generational family [11]. The following year, the first HCM-associated mutation localizing to the *MYH7*-encoded β-myosin heavy chain genetic locus was identified [12]. Since then, multiple studies have determined that the majority of HCM is due to mutations in genes encoding components of the cardiac sarcomere responsible for generating the molecular force of myocyte contraction. However, in a given cohort of unrelated patients with clinically diagnosed HCM, the frequency of mutations localizing to these genes, the so-called “sarcomere-positive” individuals, varies from approximately 25% to 65% depending on the cohort analyzed [13–16]. Worldwide, approximately 50% of patients diagnosed with HCM are sarcomeric mutation negative [17].

Sarcomeric-HCM genes are divided into sub-groups based on the type of protein encoded. These include proteins comprising the sarcomere’s thick myofilament (*MYH7*, *MYL2*-encoded regulatory myosin light chain, and *MYL3*-encoded essential myosin light chain [18,19]), intermediate myofilament (*MYPBC3*-encoded cardiac myosin binding protein C [20]), and thin myofilament (*ACTC*-encoded actin [21], *TPM1*-encoded alpha-tropomyosin [22], *TNNT2*-encoded cardiac troponin T [22], and *TNNI3*-encoded cardiac troponin I [23]). A handful of mutations in the “giant” myofilament, *TTN*-encoded cardiac titin have also been described [24, 25]. Despite early studies indicating that specific mutations held

prognostic significance, or that mutations localizing to particular sarcomeric genes might predispose to a particular HCM phenotype, there is no major HCM genotype-phenotype correlation that has been universally validated [26].

While the frequency of mutations localizing to sarcomeric genes varies slightly depending on the cohort analyzed, mutations in *MYBPC3* and *MYH7* are the two most common genetic subtypes [13, 14, 16]. Wide-spread acceptance of sarcomeric genes, with the exception of the still-emerging role of *TTN*, has led to the development of multiple clinically/commercially available genetic tests. While this developmental benchmark represents the advances that have been made in HCM research, at least 50% of the HCM population, and the majority of patients with sigmoidal and apical septal morphologies remain, genotype-negative [27–29]. This has afforded the opportunity for identification of novel genes that may be responsible for HCM and has cultivated a wealth of research to identify these still enigmatic mechanisms of cardiac hypertrophy.

Cardiac Troponin C – The Most Recent Sarcomeric Protein Implicated in HCM

The cardiac troponin heterotrimeric myofilament complex is comprised of an elongated troponin T (TNNT2) subunit, an inhibitory troponin I (TNNI3) subunit, and a Ca^{2+}-sensitive troponin C subunit (TNNC1, also denoted HcTnC) encoded by the gene *TNNC1*. While *TNNT2* and *TNNI3* were established as HCM-susceptibility genes over a decade ago, HCM-associated mutations localizing to *TNNC1* have only recently been identified [30–33]. At the molecular level, TNNC1 is a sarcomeric Ca^{2+} sensor which, when bound to the cytosolic divalent cation at the single Ca^{2+}-specific binding site, strengthens its interaction with TNNI3. This binding reduces the inhibitory function of TNNI3, releasing it from actin, and allows a shift in the troponin-tropomyosin complex which slides deeper into the actin groove exposing the myosin-binding sites. In this way, TNNC1 represents a critical molecular switch which initializes myofilament contraction [34].

The first case report identifying an HCM-associated mutation in TNNC1, L29Q, was subsequently shown to reduce TNNC1 Ca^{2+}-sensitivity by blunting the functional effects of TNNI3 phosphorylation on TNNC1 [30, 31]. While the observed reduction Ca^{2+}-sensitivity has proven controversial, it is possible that TNNC1-L29Q functionally disrupts the dynamic interaction of TNNI3 and TNNC1 [32, 33]. Subsequently, four additional mutations, TNNC1-A8V, C84Y, E134D, and D145E, were identified in a large cohort of 1025 unrelated cases diagnosed with HCM [35]. Concomitant comprehensive genetic analysis of 600 reference alleles did not identify any protein-altering genetic variation among healthy individuals. Cardiac fibers reconstituted with mutated TNNC1 demonstrated increased Ca^{2+}-sensitivity to contractile force generation with A8V, C84Y and D145E mutations. This property of increased myofilament Ca^{2+}-sensitivity fits well with the defective cardiac relaxation noted in both animal models and patients with HCM and is in contrast to dilated cardiomyopathy (DCM)-associated *TNNC1* mutations which have reduced Ca^{2+}-sensitivity and are associated with loss of contractility [36, 37]. Indeed, increased Ca^{2+}-sensitivity may be a defining characteristic of HCM-associated *TNNC1* mutations [38, 39]. Further characterization of TNNC1-A8V, C84Y, and D145E demonstrated mutation-induced alterations in protein secondary structure that modifies dynamic interactions with other cardiac thin filament proteins through potentially mutation-specific mechanisms [40]. In addition, TNNC1-D145E, and to a lesser degree E134D, may perturb Ca^{2+} association with the carboxy-terminal Ca^{2+}-binding EF hands of TNNC1 [41]. Finally, the first frameshift mutation in *TNNC1* was recently identified in a youthful proband who died of SCD [42]. This insertion results in alteration at residue Q122 leading to an out-of-frame scramble of following residues and premature protein truncation (Q122fs/30). While this mutation cosegregates with incidence of LV hypertrophy in the identified family, in-depth functional

studies delineating the potential molecular and cellular impact of this mutation are yet to be completed.

Taken together, these studies demonstrate that mutations in *TNNC1* are rare, occurring in ~0.4% of HCM patients. Despite this rarity, *TNNC1* mutations demonstrate a frequency similar to other genes included in the current sarcomeric genetic test panel including *TNNI3* (~1.5%), *TPM1* (~0.77%), and *ACTC* (~0.26%) [27]. Based on the functional, apparently HCM-specific, impact of these mutations in multiple studies, and the absence of genetic variation in ostensibly healthy controls to date, *TNNC1* has been incorporated into the HCM genetic test as an HCM-associated sarcomeric gene [43].

HCM DUE TO MUTATIONS IN NON-SARCOMERIC CALCIUM-HANDLING PROTEINS

Calcium Signaling in the Cardiocyte

A major role of Ca^{2+} within the cardiocyte is the initiation and coordination of myofilament contraction – a critical process of the cardiocyte and one that is accordingly tightly regulated (Reviewed [44, 45], Fig. 1). Opening of the voltage-gated L-type Ca^{2+} channel (LTCC) at the sarcolemma allows for an influx of extracellular Ca^{2+} across the cardiac dyad. This triggers Ca^{2+} release from the sarcoplasmic reticulum (SR) via *RYR2*-encoded ryanodine receptors type 2 (RyR2) in a process known as Ca^{2+}-induced Ca^{2+}-release (CICR). Cytosolic Ca^{2+} binds to TNNC1 of the myofilament and serves as the molecular initiator for mechanical contraction [46]. Systolic contraction is terminated by re-uptake of cytosolic Ca^{2+} into the SR via the SR Ca^{2+} ATPase (SERCA2) and removal of Ca^{2+} from the cell through the sodium-Ca^{2+} exchanger (NCX1). *PLN*-encoded phospholamban (PLN) negatively-regulates the Ca^{2+} uptake action of SERCA2. In this way, Ca^{2+} is the critical ion that couples membrane excitation with cardiocyte contraction, a process known as excitation-contraction coupling [47, 48].

Calcium-Handling Protein-Mediated HCM

Given the critical role of Ca^{2+} in excitation-contraction coupling and the expanding body of literature linking alterations in Ca^{2+}-handling with hypertrophic remodeling, investigators have begun to explore whether mutations in genes encoding Ca^{2+}-handling proteins might be associated with HCM. *TNNC1*, one of the first genes encoding a Ca^{2+} sensitive/handling protein to be firmly linked with HCM, was a logical target as it represents a Ca^{2+}-sensitive element of the cardiac sarcomere (Fig. 2A). Further, it is part of the same thin filament macromolecular complex that several other canonical HCM myofilament proteins comprise. Since this first association, mutations in several other genes, including *PLN*, *JPH2*, *RYR2*, *CASQ2*, *CALR3*, and *SRI*, have been suggested to contribute to the pathogenesis of HCM to varying degrees (Tables 2 & 3). Given the complete absence of reported genetic variation in *TNNC1* and *PLN* among healthy individuals to date, inclusion of these two genes in the clinically available genetic test may be warranted.

Phospholamban

Cardiocyte relaxation after contraction is an active process mediated by ATP-expending pumping of cytosolic Ca^{2+} into the SR lumen through the action of the ATPase SERCA2 or the exchange of Ca^{2+} out of the cell for Na^{+} through the function of NCX1. PLN is a highly regulated small protein binding partner of SERCA2 which, when bound, inhibits the Ca^{2+}-reuptake action of SERCA2. This inhibitory action can be relieved by phosphorylation, and PLN serves as a substrate for the kinase-action of protein kinase A or calmodulin-dependent

protein kinase 2 [49, 50]. Inhibition of PLN results in increased SERCA2-mediated Ca^{2+} removal from the cytosol and increased diastolic relaxation.

Rare mutations and common polymorphisms localizing to both the promoter and coding region of *PLN* have been associated with HCM (Fig. 2B). A handful of rare promoter variants have been identified in multiple independent cohorts of HCM patients including a C to T conversion at position −235 (C>T −235), A>C −198, A>G −120, T>C −114 [51], A>G −77 [52], G>T −47 (2 probands) [51], and C>G −42 [53]. Each of these rare variants was not found in ethnically-matched controls. Two of these probands, C>T −235 and T>C −144, hosted compound mutations in *MYH7* and *MYL2*, respectively.

In addition to promoter variants, a mutation in the coding region of *PLN* has been associated with HCM. The nonsense mutation, PLN-L39X, was originally identified in a DCM proband and was found to cosegregate with incidence of both DCM and HCM in a large family [54]. Within this family, kindred hosting a homozygous L39X mutation demonstrated, or quickly progressed to, DCM/heart failure, while individuals hosting a heterozygous mutation were unaffected or demonstrated HCM. Subsequently, this mutation has been identified in two HCM probands from two independent cohorts of HCM index cases [51, 55]. In one study, the L39X mutation cosegregated in a small family demonstrating a solely HCM phenotype. To our knowledge, L39X represents the only HCM-associated *PLN* mutation which alters the protein sequence and may represent an HCM-associated mutation “hot spot” within the PLN primary sequence.

Overall, among studies exploring index case cohorts of HCM, *PLN* promoter and coding-region mutations were identified in ~0.50% of HCM probands which is similar to the frequency of *TNNC1*, *TPM1*, and *ACTC*-associated HCM mutations [27, 35]. As with *TNNC1*, comprehensive genetic interrogation of 600 reference alleles did not identify any rare promoter variants or amino acid substitutions which might be considered “false positive” results for genetic testing. Given these findings, as well as the relatively small size of the *PLN* genetic locus, inclusion of *PLN* on the genetic test for HCM appears reasonable.

Junctophilin 2

Junctophilins (JPHs, also known as JPs) are a family of proteins found in all excitable cells from striated muscle to neurons [56, 57]. JPHs contain multiple amino-terminal membrane occupation and recognition nexus (MORN) motifs which localize the amino-terminus of protein to the plasma membrane. A carboxy-terminal membrane-spanning domain embeds the opposing end into the ER/SR [58, 59]. In this way, through bridging the subcellular space between the plasma membrane and the ER/SR, JPHs play a critical role in the maintenance of effective Ca^{2+}-handling.

JPH2 is the major JPH family member in the heart and plays a key role in maintaining the critical ultrastructural geometry of the cardiac dyad needed for effective CICR [60]. JPH2 null mice are embryonic lethal and demonstrate alteration of the cardiac dyad distance as well as vacuolization of the SR. Cardiocytes demonstrate stochastic contraction and irregular, smaller Ca^{2+} transients [59]. Rodent models of HCM, DCM, and pressure-induced hypertrophy/heart failure are associated with loss of JPH2 expression [61–63]. Recently, this observation of reduced JPH2 expression has been extended to humans with HCM. Expression silencing of JPH2 *in vitro* is sufficient to cause cellular hypertrophy and reduced CICR [64]. *In vivo* conditional knock-down JPH2 mice rapidly develop heart failure with disrupted cellular architecture and reduced excitation-contraction coupling gain [65].

Three mutations localizing to the amino terminus of JPH2 have been associated with a largely Caucasian HCM cohort of 388 individuals – S101R, Y141H, and S165F [66] (Fig.

2C). These mutations, localizing to the first MORN motif domain and the linker domain, were identified in three unrelated individuals negative for sarcomeric or Z-disc mutations. Each of these mutations were found to reduce CICR amplitude and disrupt cellular ultrastructure utilizing *in vitro* myocyte models, and in the case of Y141H and S165F, induce cellular hypertrophy [66]. These findings are in close agreement with the increase in cell size, induction of pro-hypertrophic transcriptional markers, and reduced CICR seen with JPH2 expression knock-down [64]. Subsequent studies in skeletal muscle found that the S165 residue is part of the reciprocal binding domain for the transient receptor potential cation channel 3 (TRPC3), a non-selective cation channel in the plasma membrane which allows for influx of extracellular Ca^{2+} and Na^{+} to enter the cell [67–69]. Phylogenic and biochemical analyses identified the S165 residue as a protein kinase C phosphorylation site which is abolished by the S165F mutation resulting in reduced binding to TRPC3, decreased CICR, and hypertrophy in skeletal myocytes [57, 70].

Two additional genetic variants localizing to the divergent domain, JPH2-R436C and G505S, have been reported in two and four unrelated individuals, respectively, within an independent Japanese HCM cohort [71]. Importantly, the R436C variant was also identified in two ostensibly healthy Japanese control individuals in the study and may represent a polymorphism rather than a true pathogenic mutation [71]. While the G505S variant was not originally identified in the healthy control Japanese cohort, we have identified this variant in ~0.5% of ostensibly healthy Caucasian Americans and ~2.0% of African Americans (unpublished). As is the case with the R436C variant, given the ~0.2% frequency of HCM in the general population and the relative rarity of non-sarcomeric gene mutations, G505S is unlikely to be a pathogenic mutation in isolation.

Overall, these studies indicate that mutations in *JPH2* are a rare cause of HCM, found in less than 1% of index cases. Due to the presence of both common and rare genetic variation at this genetic locus in reference alleles, inclusion of *JPH2* on the HCM genetic test panel at this time is likely to make interpretation of a positive test challenging. Despite this practical consideration, these mutations have catalyzed molecular insight into an enigmatic protein with a clear physiologic and pathophysiologic role in both cardiac and skeletal muscle. Additional studies are needed to further clarify the role of JPH2 dysregulation in hypertrophic remodeling as well as to elucidate the potential role of perturbed JPH2 in other cardiovascular diseases.

Ryanodine Receptor 2

The major molecule responsible for CICR and Ca^{2+}-mediated myofilament contraction is RyR2. Heritable mutations in *RYR2* have been associated with catecholaminergic polymorphic ventricular tachycardia (CPVT) [72, 73], sudden infant death syndrome [74], and arrhythmogenic right ventricular cardiomyopathy/dysplasia [75]. While it has been hypothesized to be a rare cause of HCM by many investigators, there has not been a definitive study associating mutations in *RYR2* with HCM. To our knowledge, the only HCM-associated mutation described to date is the RyR2-T1107M mutation. Originally identified in an individual negative for sarcomeric mutations, this mutation was absent in ostensibly healthy controls and co-segregated with incidence of HCM upon pedigree analysis [76]. Interestingly, this proband also demonstrated a clinical history of ventricular fibrillation and this variant has been previously described in a cohort of CPVT cases [77]. In addition to this study, a recent linkage study involving a large family with genotype-negative HCM identified a locus containing the gene for alpha actinin 2 (*ACTN2*) and *RYR2* [78]. While mutations in *ACTN2* were found to co-segregate in this study, it is possible that compound mutations in the over 60 *RYR2* coding exons not genotyped in this study may still exist.

Calsequestrin 2 and Calreticulin 3

RyR2-mediated SR Ca^{2+}-release is a highly regulated event and several modulators, both cytosolic and SR luminal, have been shown to alter RyR2 gating. While the carboxy-terminal domain of each RyR2 monomer comes together to form a central pore, RyR2 has a large cytosolic amino-terminus which modulates its Ca^{2+}-gating action. The cytosolic domain has multiple phosphorylation sites as well as binding sites for various interacting proteins which can either increase or decrease channel opening probability [79–84]. Similarly, the luminal side of RyR2 is regulated by modulatory proteins which are critical for the proper functioning of RyR2.

CASQ2-encoded calsequestrin 2 (CASQ2) serves as a low-affinity, high-capacity Ca^{2+} buffer within the SR [85]. While its role in modulating cellular Ca^{2+} homeostasis is unclear, it is known that CASQ2 functions as a Ca^{2+} sensor for RyR2 as well as a modulator of free Ca^{2+} levels in the store. Over-expression of CASQ2 has been linked to increased SR luminal Ca^{2+} load and induction of cardiac hypertrophy in animal models [86, 87]. While only a handful of *CASQ2* mutations have been reported, nearly all have been associated with the development of CPVT in cases negative for mutations in *RYR2* [88, 89]. To our knowledge, only a single *CASQ2* mutation, D63E, has loosely been associated with HCM [55]. The small family found to be positive for CASQ2-D63E also hosted two compound mutations in the *MYBPC3* (MYPBC3-R326Q and Q1233X) which also co-segregated with incidence of disease. The presence of these compound mutations provide an alternative genetic explanation for the pathogenesis of HCM in this family and thus decrease the candidacy of CASQ2-D63E as a HCM-causing mutation.

CALR3-encoded calreticulin 3 (CALR3) is another SR luminal protein which serves as a Ca^{2+}-buffering chaperone that regulates SR store Ca^{2+} levels as well as modulates function of various Ca^{2+} channels and pumps [90]. Two mutations in *CALR3*, R73Q and K82R, have been identified in two unrelated probands with HCM within a cohort [55]. As with the CASQ2-D63E mutation, CALR3-R73Q was identified in a proband who also hosted two mutations in *MYBPC3* (D745G and P873H) which clouds any association between this particular *CALR3* variant and the development of HCM. Among these three identified mutations, only CALR3-K82R was identified in a proband negative for the mutations in the canonical sarcomeric genes.

Sorcin

SRI-encoded sorcin is a member of the penta-EF hand protein family which undergoes conformational change in response to Ca^{2+} allowing it to translocate to membranes, such as the t-tubular sarcolemma and SR, and dynamically modulate CICR [91, 92]. Sorcin has been shown to interact with and modulate several proteins, including LTCC and RyR2, and plays a role in providing negative feedback for the termination of CICR [93, 94]. The SRI-F112L mutation was initially identified in two unrelated families with HCM and hypertension and was found to be absent in 200 control individuals [95]. However, subsequent large genomic/bioinformatic studies have identified this variant in ostensibly healthy individuals, and this variant has been generally accepted as a polymorphism [96, 97]. Further, mice over-expressing sorcin F112L did not develop LV hypertrophy or systemic hypertension, demonstrating only mild LV chamber dilatation with intact contractility [98]. Despite this, F112L disrupts the tertiary structure of the protein by X-ray crystal structure analysis resulting in reduced inhibitory function of the protein on CICR [98–100]. These results support the possibility that sorcin-F112L in isolation is not associated with the pathogenesis of HCM. It is possible that this mutation creates a genetic “second hit” in which HCM and hypertension develop due to alterations in CICR in concert with another HCM-associated mutation. Additional studies examining *SRI* in large cohorts of HCM index cases may yield

true disease-associated mutations which perturb the regulatory function of sorcin in a pro-hypertrophic manner.

FUTURE DIRECTIONS

While real progress has been made in identifying the cause of individuals with HCM who lack sarcomeric gene mutations, alternative genetic subtypes still account for only a small subset of these patients. Additional candidate gene analyses may be useful in elucidating those individuals hosting mutations localizing to proteins that are already known to influence hypertrophic remodeling of the heart. In particular, two Ca^{2+}-sensitive pro-hypertrophic cascades centered around calmodulin (CaM)-dependent protein kinase 2 (CaMKII) and the phosphatase calcineurin are rational targets [101–103]. The Ca^{2+}/CaM-dependent CaMKII can phosphorylate and inhibit histone deacetylase 4 (HDAC4), a repressor of hypertrophic remodeling. This repression removes the tonic inhibition of HDAC4 on the pro-hypertrophic transcription factor MEF2 [104–106]. In a similar pathway, calcineurin, a Ca^{2+}-/CaM-dependent protein phosphatase, induces cardiocyte hypertrophy through activation of the transcription factor NFAT3 and its cofactor GATA4 which colocalize to the nucleus to activate a similar pro-hypertrophic gene profile [107, 108].

In addition to the candidate gene approach, linkage analysis still has a role in determining familial HCM. While most large families with HCM have likely been subjected to linkage analysis already, there are still occasional robust reports of this technique used to identify novel genetic loci of disease association. Perhaps more sensitive techniques utilizing this same methodological principal, such as genome-wide association studies (GWAS) or whole exome sequencing, will identify novel causes of HCM pathogenesis. Each of these techniques allow for an unbiased examination of the entire genome or transcribed exome. These techniques are not limited to small genetic loci like “first generation” sequencing and are more informative for relatively less cost than “next generation” sequencing modalities. These methods also provide the opportunity to examine regions of the genome that are transcribed but not translated into protein (i.e. 5′ and 3′ untranslated gene regions or genes of non-coding RNAs) and, in the case of GWAS analysis, introns and large intergenic regions as well. While a large number of cardiovascular disease GWAS studies have been conducted to date, few disease-locus associations have been replicated and fewer still have become “actionable” biomarkers which play a clear role in clinical evaluation or management of a patient [109]. Perhaps the comprehensive analyses of transcribed genetic loci afforded by whole exome sequencing will aid in the identification of disease-associated genetic perturbations which have evaded current GWAS.

CONCLUSIONS

HCM is relatively common heritable cardiovascular disease defined by marked genotypic and phenotypic heterogeneity. While mutations in the canonical genes encoding elements of the cardiac sarcomere represent the most common genetic subtypes of HCM, mutations in genes encoding Ca^{2+}-handling proteins have been identified in some patients that are negative for the clinically available HCM genetic test. While these mutations are rare, identification of the mutations has shed light on the physiology of the cardiocyte, and when perturbed, the development of Ca^{2+}-mediated pathologic remodeling. Absence of genetic “background noise” and independent identification of mutations in various HCM cohorts argue favorably for incorporation of *TNNC1* and *PLN* into clinical genetic testing. Additional investigation into these genes, as well as identification of novel Ca^{2+}-handling protein-encoding genes associated with HCM, is warranted to uncover novel mechanisms of HCM pathogenesis.

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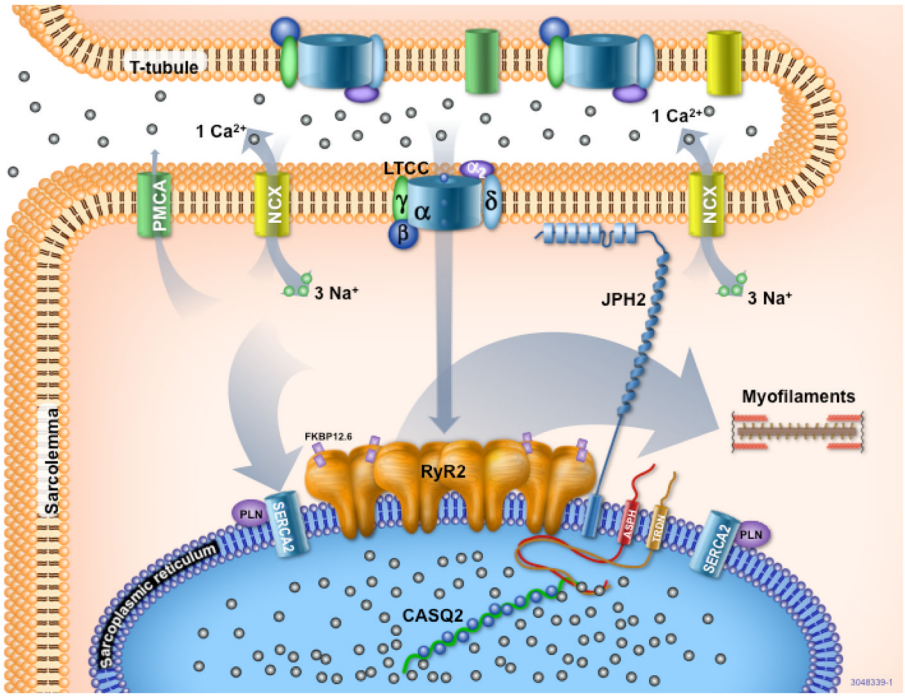

Fig. (1). Cardiocyte calcium-induced calcium-release

Ca^{2+} influx through sarcolemmal LTCCs triggers a large release of SR-stored Ca^{2+} by RyR2 which then triggers Ca^{2+}-sensitive myofilament contraction. Contraction is terminated by Ca^{2+} uptake into the SR via SERCA2 or expulsion of Ca^{2+} into the extracellular space by NCX and PMCA. Blue circles represent Ca^{2+} while green circles represent Na^{+}. Width of blue arrows is roughly proportional to the relative degree of Ca^{2+} flux.

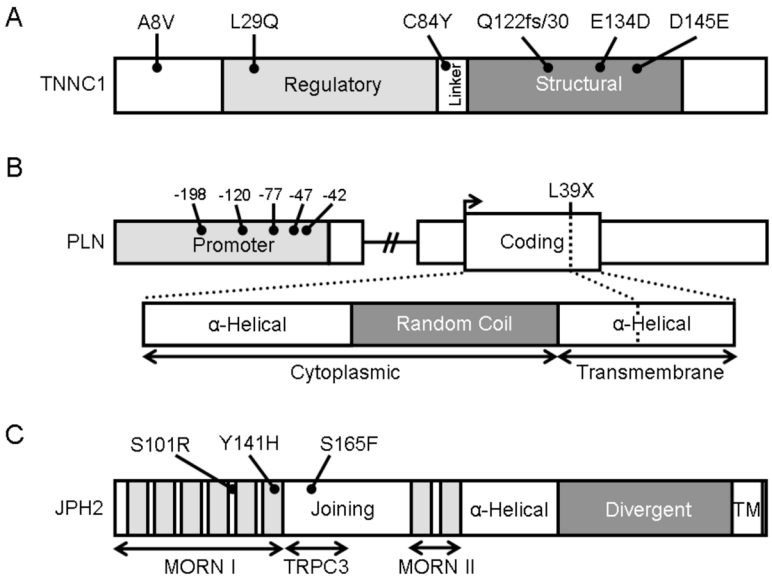

Fig. (2). Protein topology with all HCM-associated mutations for TNNC1, PLN, and JPH2

A. Linear topology of the 6 exon-encoded, 161 amino acid TNNC1. Domains include the regulatory EF-hand (light gray), the linker, and the structural EF-hand domain (dark gray). **B.** Schematic of the proximal promoter (light gray), coding sequence, and protein topology of the 1 exon-encoded, 52 amino acid PLN. Domains of PLN include two α-helical domains separated by random coil (dark gray). The amino-terminal aspect of the protein is cytoplasmic while the carboxy-terminus is a hydrophobic, SR-associated domain. **C.** Linear topology of the 5 exon-encoded, 696 amino acid JPH2. Domains include the MORN I and II domains which each contain multiple MORN motifs (light gray) and are separated by the joining region domain. Contained within the joining region is the TRPC3 reciprocal binding domain identified in rodent models of skeletal muscle. A putative α-helical domain connects the MORN II domain with the divergent domain (dark gray) and a carboxy-terminal transmembrane domain (TM) associates with the SR.

Table 1

Summary of the Genetic Causes of HCM

Gene	Locus	Protein	Frequency
Myofilament/Sarcomeric HCM			
Giant Filament			
TTN	2q31	Titin	Rare
Thick Filament			
MYH7	14q11.2-q12	β-Myosin heavy chain	15–25%
MYH6	14q11.2-q12	α-Myosin heavy chain	Rare
MYL2	12q23-q24.3	Regulatory myosin light chain	Rare
MYL3	3p21.2-p21.3	Essential myosin light chain	Rare
Intermediate Filament			
MYBPC3	11p11.2	Cardiac myosin-binding protein C	15–25%
Thin Filament			
TNNT2	1q32	Cardiac troponin T	3–5%
TNNI3	19p13.4	Cardiac troponin I	1–5%
TPM1	15q22.1	α-Tropomyosin	1–5%
ACTC	15q14	α-Cardiac actin	Rare
TNNC1	3p21.1	Cardiac troponin C	Rare
Z-Disc HCM			
ACTN2	1q42-q43	α-Actinin 2	Rare
ANKRD1	10q23.31	Cardiac ankyrin repeat protein	Rare
CSRP3	11p15.1	Muscle LIM protein	Rare
LBD3	10q22.2-q23.3	LIM binding domain 3	Rare
MYOZ2	4q26-q27	Myozenin 2	Rare
TCAP	17q12-q21.1	Telethonin	Rare
VCL	10q22.1-q23	Vinculin/metavinculin	Rare
Calcium-Handling HCM			
CALR3	19p13.11	Calreticulin 3	Rare
*CASQ2**	1p13.3-p11	Calsequestrin	Rare
JPH2	20q13.12	Junctophilin 2	Rare
PLN	6q22.1	Phospholamban	Rare
*RYR2**	1q43	Ryanodine receptor 2	Rare
*SRI**	7q21.1	Sorcin	Rare

Asterisk indicates additional studies may be needed before accepting as a rare HCM-susceptibility gene. Frequency estimates are derived from a population of all unrelated patients with clinically diagnosed HCM without further clinical refinement based on family history, degree of hypertrophy, age at diagnosis, or septal profile. Rare indicates <1% contribution.

Table 2

Summary of Possible HCM-Associated Mutations Involving Calcium-Handling Proteins

Mutation
Troponin C
A8V
L29Q
C84Y
Q122fs/30
E134D
D145E
Junctophilin 2
S101R
Y141H
S165F
(R436C)
(G505S)
Ryanodine Receptor 2
(T1107M)
Calsequestrin 2
(D63E)
Calreticulin 3
(R73Q)
K82R
Sorcin
(F112L)
Phospholamban
(C>T −235)
A>C −198
A>G −120
(T>C −114)
A>G −77
G>T −47*
C>G −42
L39X*

Parentheses indicate compound sarcomeric HCM-associated mutations identified in the proband, identification of the mutation in ostensibly healthy individuals, or unpublished.

*, two mutation-positive probands identified.

Table 3

Summary of Cohort Demographics

Demographic/Feature	HCM	Myofilament	Calcium-Handling Proteins			
			Overall	TNNC1	PLN	JPH2
No. of probands	388	186	17	6	8	3
Proportion of cohort†	100%	47.8%	1.64%	0.40%	0.47%	0.77%
Proportion female	44.7	44.6%	35.3%	16.7%	50.0%	33.3%
Age at diagnosis (yrs)	41.2 ± 19	35.5 ± 16	41.3 ± 4	32.3 ± 9	53.5 ± 4	27.0 ± 2
Maximum LVWT (mm)	21.3 ± 6	22.8 ± 6	20.9 ± 1	19.5 ± 2	20.1 ± 2	26.0 ± 6
Mean LVOTO (mmHg)	44.5 ± 42	41.6 ± 41	34.0 ± 11	61.8 ± 21	21.9 ± 17	20.3 ± 11
Family history of HCM	35.2%	48.9%	47.1%	50.0%	50.0%	33.3%
Family history of SCD	21.2%	27.7%	11.8%	33.3%‡	0%	0%
Myectomy	44.6%	49.1%	35.3%	50.0%	25.0%	33.3%
ICD	20.2%	29.4%	23.5%	0%	25.0%	66.7%
Pacemaker	24.9%	24.6%	11.8%	0%	12.5%	33.3%

No. of probands, the number of mutations-positive probands in a cohort of Mayo Clinic patients (HCM, Myofilament columns) and all mutation-positive probands not hosting compound sarcomeric mutations identified in the literature (Calcium-Handling Proteins columns).

†, proportion of the Mayo Clinic HCM cohort; LVWT, maximum left ventricular wall thickness; LVOTO, left ventricular outflow tract obstruction; SCD, sudden cardiac death;

‡, SCD of two probands reported; ICD, implantable cardioverter defibrillator.