FOCUS SEMINAR: GENETICS

STATE-OF-THE-ART REVIEW

Clinical and Mechanistic Insights Into the Genetics of Cardiomyopathy

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ABSTRACT

Over the last quarter-century, there has been tremendous progress in genetics research that has defined molecular causes for cardiomyopathies. More than a thousand mutations have been identified in many genes with varying ontologies, therein indicating the diverse molecules and pathways that cause hypertrophic, dilated, restrictive, and arrhythmogenic cardiomyopathies. Translation of this research to the clinic via genetic testing can precisely group affected patients according to molecular etiology, and identify individuals without evidence of disease who are at high risk for developing cardiomyopathy. These advances provide insights into the earliest manifestations of cardiomyopathy and help to define the molecular pathophysiological basis for cardiac remodeling. Although these efforts remain incomplete, new genomic technologies and analytic strategies provide unparalleled opportunities to fully explore the genetic architecture of cardiomyopathies. Such data hold the promise that mutation-specific pathophysiology will uncover novel therapeutic targets, and herald the beginning of precision therapy for cardiomyopathy patients. (J Am Coll Cardiol 2016;68:2871–86) © 2016 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Cardiomyopathies are disorders with primary abnormalities in the structure and function of the heart. Addition of the historical term *idiopathic* was used to denote an enigmatic etiology that specifically excluded pre-existing cardiovascular or systemic diseases. These disorders are commonly grouped into morphological subtypes that include hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and left ventricular noncompaction (LVNC) (1,2). Early clinical investigations recognized familial transmission for many cardiomyopathies, suggesting a critical role for genetics. Research advances over the past 30 years confirmed this hypothesis, and today many cardiomyopathies are recognized as monogenic disorders.

Cardiomyopathy mutations are notable for substantial variation in clinical expression. Both genetic heterogeneity (e.g., distinct genes that cause the same disease) and allelic variation (distinct mutations in the same gene) contribute to variable morphological phenotypes and disease severity. Background genomic variation, lifestyle, and exposures are other contributory factors that account for differences in clinical manifestations in family members with identical mutations. These complexities pose substantial challenges for clinicians faced with

interpreting genetic testing results and effectively using this information to improve patient management, as well as for scientists trying to decipher disease mechanisms. Herein, we review the current understanding of cardiomyopathy genetics, and discuss how the rapid evolution of genomic technologies is changing this landscape at both the bench and the bedside.

DEFINING PATHOGENIC VARIANTS

The era of genetic medicine in cardiomyopathy research began with studies that used genome-wide linkage analysis to identify mutations that displayed classic Mendelian inheritance (3). These studies surveyed all chromosomes, and then restricted analyses of genes and variants using familial cosegregation to achieve strong statistical evidence that an identified candidate gene was likely to cause disease. Additional evidence included expression of the candidate gene in the disease tissue, demonstration that the mutation was absent from the general population, and that it altered an amino-acid residue that was highly conserved across species, thus implying functional consequences. Definitive pathogenic confirmation required the identification of additional mutations in the same gene via analyses of unrelated affected patients or demonstration that the human phenotype was recapitulated by expression of the variant in a model system (**Table 1**). This overarching strategy has now been supplemented with direct sequence analyses using arrays or next-generation sequencing to study the exome (sequences that encode all proteins) and genome.

Core sets of pathogenic cardiomyopathy genes have been successfully identified, but knowledge of the full genetic architecture remains incomplete. Novel disease genes have been reported in association with a cardiomyopathy, but with less definitive proof of pathogenicity. This can reflect small family size that limits statistical power, incomplete segregation analyses due to sample access, and the considerable effort and expense required for comprehensive study of all family members, or reliance on predicted pathogenicity on the basis of variant characteristics. Variants that cause major structural changes in the encoded protein (truncating, nonsense, and canonical splice-site mutations) are usually considered more likely pathogenic than missense mutations that alter 1 amino acid, but neither assumption is uniformly true. The development of in silico algorithms has improved the prediction of pathogenicity on the basis of expected

TABLE 1 Criteria for Defining a Variant as Pathogenic

Criterion	Description and Significance
Nonsynonymous genetic variant	A genetic variant that alters the amino-acid sequence
Expression	Confirmation that the protein product of the gene is expressed in diseased tissue
Unbiased genetic analysis	Genome-wide analysis (linkage analysis, genome-wide association study, whole-exome or whole-genome sequencing) reduces the possibility of identifying a coincidental and unrelated mutation or a disease-modifying mutation
Conservation	Conservation of the affected amino acid in different species through evolution suggests a critical biological role of that particular residue
Control population	Absence from a large number of unaffected controls, or confirmation of very low minor allele frequency in a population of interest demonstrates that the mutation is unlikely to be a polymorphism unrelated to the disease
Cosegregation	Demonstrates that the mutation is found in those with disease only (note: incomplete and age-dependent penetrance is common); identification of the disease phenotype in those without the mutation proves nonpathogenicity
Multiplicity of events	Demonstration that a variant may be causal in ≥2 unrelated probands greatly increases confidence in pathogenicity
Animal models	Recapitulation of the disease phenotype by the variant in animals genetically engineered to express the mutation strongly supports causality

protein structural changes, evolutionary conservation of amino acids across protein orthologs, knowledge of protein function, and altered messenger ribonucleic acid transcript splicing. But even with these tools, the specificity is low and overall accuracy is only ∼65% to 80% for predicting pathogenicity (4).

A recently developed and publicly available resource, the Exome Aggregation Consortium (ExAC) (5) has further improved variant classification. ExAC data summarize the individual frequencies of all variants identified in exome sequences (which encode all proteins in the genome) from over 60,000 unrelated individuals. Notably, the frequency of specific variants differs among peoples of different ancestries. On the basis of the assumption that pathogenic variants will be very rare or absent in a general population with shared ancestry, the ExAC frequency provides a benchmark for whether or not a variant is likely to cause cardiomyopathy. Already this dataset has provided supportive evidence that prioritized certain variants for further testing, while also casting doubt on the pathogenicity of some cardiomyopathy-associated variants that were identified by candidate gene analyses (6). Further expansion of this database to include many more

distinct populations will continue to improve variant interpretation.

The genetic diversity that underlies the cardiomyopathies remains a complex issue. Next-generation sequence analyses of disease and control cohorts have demonstrated that 60% to 90% of rare cardiomyopathy mutations are "private" and found only in single families (7,8). In addition, haplotype analyses of identical mutations in separate families have confirmed that most are recent and independent mutational events (9). Consequently, clinical genetic testing results often contain a nuanced interpretation, rather than definitively labeling a variant as pathogenic or benign (**Table 2**).

GENETIC CARDIOMYOPATHIES

HYPERTROPHIC CARDIOMYOPATHY. HCM is a primary disorder of the heart that is unaccompanied by pathology in another organ (10). The pathological hallmark of HCM is unexplained left ventricular hypertrophy (LVH). Diagnosis requires exclusion of secondary causes of LVH, such as hypertension and aortic stenosis, or physiological hypertrophy, as seen in highly trained athletes. Classically, HCM results in asymmetric septal hypertrophy, although almost any pattern of LVH can be associated with the disease (11,12).

HCM is a monogenic disorder caused by mutations in genes that encode the protein components of the cardiac sarcomere (**Figure 1**, Online Table 1). Clinical studies define the population prevalence for unexplained LVH as 1:500 (13,14), which is far higher than the prevalence of pathogenic mutations. However, next-generation sequence analyses of sarcomere genes have identified considerable numbers of rare variants in population cohorts, including among individuals with some clinical features of cardiomyopathy. Together, these data imply that variants in sarcomere genes may individually or collectively affect cardiac morphology and function, even without causing overt HCM (8,15-17).

HCM is transmitted as a dominant trait; hence, first-degree relatives of affected individuals have a 50% risk of developing disease. However, penetrance is incomplete and lowest at very young ages, which often delays clinical diagnosis until adolescence or adulthood. Identical HCM mutations can produce distinct LVH morphologies, varying amounts of myocardial fibrosis, and differing susceptibility to arrhythmias. Genetic modifiers, epigenetic differences, and distinct environmental factors are likely to influence these variables.

HCM genes. Multiple independent studies have demonstrated pathogenic mutations causing HCM in

TABLE 2	Variant Classification
Designation	**Definition**
Pathogenic	Variants with very low population frequency (i.e., MAF <0.02%) and with strong evidence for cosegregation with disease phenotype, conservation across species, or functional evidence
Likely Pathogenic	Variants with very low population frequency (i.e., MAF <0.02%) in the population, but with lower strength for cosegregation or limited functional evidence
Variant of uncertain significance	Variant with restricted evidence for cosegregation, limited or contradictory functional evidence, and variable conservation across species
Likely benign	Variant identified at low frequency in the general population (i.e., MAF >0.3%) and not conserved across species
Benign	Variant identified at moderate levels in the general population (i.e., MAF >1%)

Adapted with permission from Richards et al. (4).
MAF = minor allele frequency.

8 genes: β-myosin heavy chain (*MYH7*) (18,19), α-tropomyosin (*TPM1*), cardiac troponin T (*TNNT2*) (20,21), cardiac myosin binding protein-C (*MYBPC3*) (22,23), myosin regulatory light chain (*MYL2*), myosin essential light chain (*MYL3*) (24), cardiac troponin I (*TNNI3*) (25), and cardiac α-actin (*ACTC1*) (26,27). Combined, *MYBPC3* and *MYH7* account for up to 50% of all clinically recognized cases of HCM, and constitute at least 75% of probands where a mutation is identified, whereas other HCM genes account for <10% of cases (28-33). Disease-causing mutations are identified in ~30% to 60% of probands in studies that include these 8 pathogenic HCM genes, (28,29,34,35), with the highest detection rate among probands with: 1) early onset disease; 2) greater severity of LVH; 3) asymmetric septal hypertrophy; and 4) family history of HCM (34,36,37).

Mutations in other genes have been associated with HCM. Unbiased genome-wide studies on 2 separate families have identified mutations in myozenin 2 (*MYOZ2*) (38) and α-actinin 2 (*ACTN2*) (39), molecules that interact with the sarcomere, but are not directly involved in force generation. Although mutations in these genes are likely pathogenic, identification of additional families or evidence that animal models carrying these mutations cause disease is needed to support their pathogenicity in HCM. In contrast, mutations in *TTN*, encoding the giant protein titin, are infrequently reported in HCM patients (40), and the pathogenicity of these mutations has been contested by multiple subsequent studies (29,41,42). Similarly, identical mutations in *CSRP3*, encoding the Z-disc molecule cardiac LIM protein, have been associated

FIGURE 1   Hypertrophic Cardiomyopathy Disease Genes

A schematic of definitive **(bold)** and posited hypertrophic cardiomyopathy genes with the subcellular localization of the encoded proteins. All pathogenic genes encode sarcomere proteins. Putative hypertrophic cardiomyopathy genes encode these and sarcomere-associated molecules.
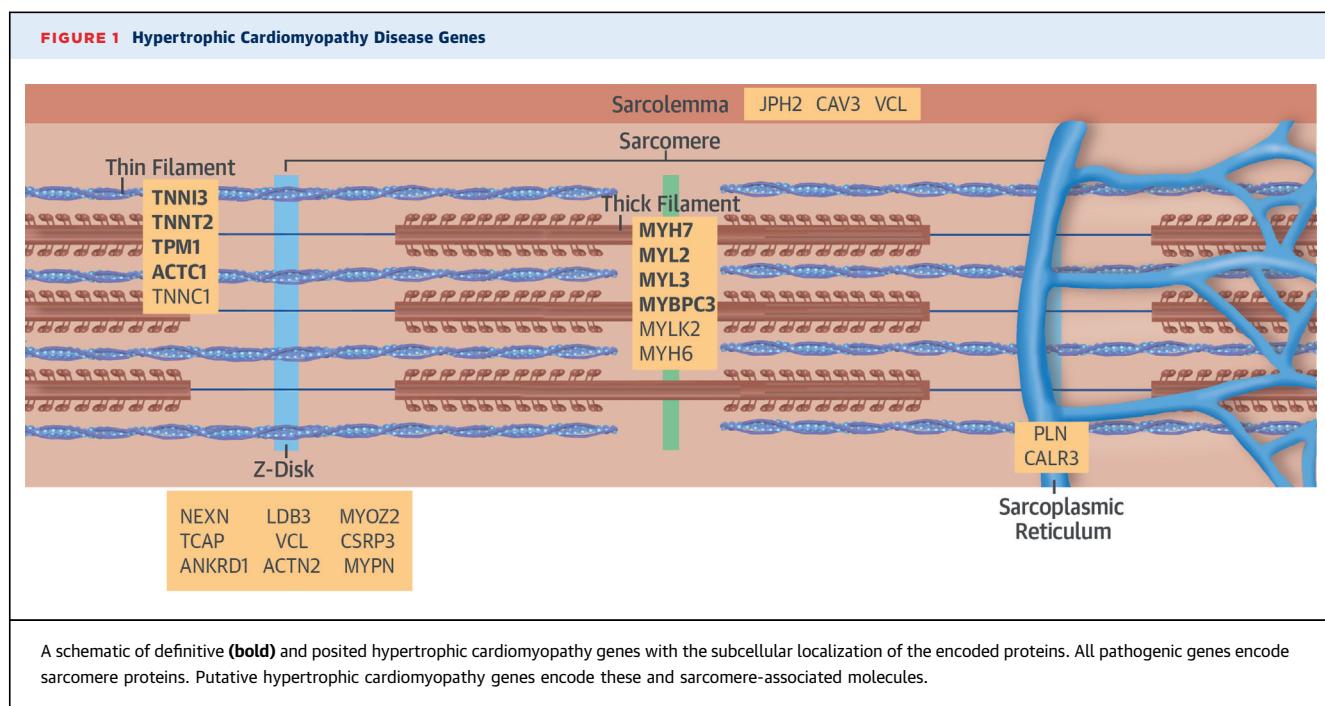
with both HCM and DCM through candidate gene analyses in separate cohorts (43-45).

Functional consequences of HCM mutations. Although the genetic basis for a large proportion of HCM is well established, the biochemical and biophysical mechanisms by which sarcomere gene mutations cause disease remains incompletely understood. To inform these processes, HCM mutations have been engineered into mice to permit detailed biochemical and molecular analyses. Although informative, interpretation of these models is complicated by the predominant expression of similar, but nonidentical myosin heavy chain isoforms (human: β-myosin; mouse: α-myosin), and differences in calcium homeostasis and cardiac physiology (e.g., the 10-fold difference in human and mouse heart rates). Despite these complexities, mouse models of HCM reveal unexpected consequences of how dominant mutant proteins that are incorporated into myofilaments perturb sarcomere function.

The myosin head region is the domain that generates force, hydrolyzes adenosine triphosphate and interacts with regulatory light chain, essential light chain, troponin T, and actin. Mutations in this domain exhibit biophysical properties that enhance contraction, but impair relaxation. Studies of isolated myofibrillar tissue preparations from these models and assessment of the chemo-mechanical cycle in ex vivo preparations of sarcomere proteins with HCM mutations confirm these observations (46).

Consistent with this mechanism, MYK-461, a small molecule that inhibits the myosin adenosine triphosphatase (ATPase) and reduces sarcomere power, was shown to attenuate the development of HCM in mice that carry pathogenic myosin mutations (47).

Far less is known about how *MYBPC3* mutations cause HCM. Myosin binding protein-C does not directly participate in force generation, but modulates contractile performance through interaction with myosin and titin. HCM mutations in other genes encode missense mutations that alter only 1 amino acid. In contrast, many *MYBPC3* mutations produce truncated proteins (48). If incorporated into the sarcomere, these mutant proteins could cause disease. Alternatively, if mutant transcripts or proteins are cleared by cell surveillance mechanisms, disease would result from haploinsufficiency, an inadequate amount of functional myosin binding protein-C for normal sarcomere performance (49-51).

In addition to the biophysical consequences on sarcomere performance, HCM mutations increase the energetic cost of contraction. Cardiac tissues from humans and model organisms show a reduced phosphorylated creatinine to adenosine triphosphate ratio, altered ATPase activity, and an overall increased energetic cost of cross-bridge cycling (52-56). These data suggest that perturbation of myocardial energetics is a common underlying molecular feature of HCM, which may account for the similar phenotypes derived from these different mutations (53,56).

Genotype-phenotype correlation. The large numbers of pathogenic mutations in different sarcomere protein genes, combined with modifying genetic, epigenetic, and environmental factors, account for why genotype alone cannot predict patient-specific outcomes in HCM. Despite this, clinical data from large families with specific mutations have provided some noteworthy insights into how genotype can impact phenotype.

Adverse clinical outcomes have been identified in several recurrent *MYH7* mutations, each of which causes substantial cardiac remodeling (Arg403Gln, associated with increased risk for sudden cardiac death; Arg719Trp and Arg453Cys, associated with increased risk for end-stage heart failure [HF]), whereas other *MYH7* mutations produce milder phenotypes and have a good prognosis (57-59). HCM caused by *MYBPC3* mutations generally presents later in life, and displays less morbidity and lower penetrance (60,61). Supporting this, HCM founder mutations occur almost exclusively in *MYBPC3* (62). Two such mutations are particularly illustrative. Among individuals with Southeast Asian ancestry, ~4% of the population carries a *MYBPC3* mutation that is estimated to have arisen ~30,000 years ago (63). This mutation is associated with a 7-fold increased risk of HF late in life. Another *MYBPC3* founder mutation dating to the 15th century today accounts for nearly 60% of HCM in Iceland, and is associated with adverse outcomes later in life (64). Maintenance of such variants through hundreds to thousands of years and generations would only occur via neutral selection with no impact during childbearing years; only recently, with increased life expectancy, has disease become apparent.

STORAGE AND METABOLIC CARDIOMYOPATHIES. Mutations in genes that encode proteins that function in cardiac metabolism or clearance of cellular byproducts also cause LVH (Online Table 2). Although these are often labeled as HCM phenocopies on the basis of cardiac imaging findings that mimic HCM, histopathology demonstrates the absence of cardiomyocyte disarray and the presence of cytoplasmic vacuoles that contain lipid (*GLA* mutations), lysosomal remnants (*LAMP2* mutations), and glycogen (*PRKAG2* and *GAA* mutations). These cardiomyopathies have distinct modes of inheritance, including autosomal-dominant (*PRKAG2* mutations), *X*-linked (*GLA* and *LAMP2* mutations), and autosomal-recessive patterns (*GAA* mutations).

Gene-based diagnosis, unlike cardiac imaging, can readily distinguish these disorders from HCM, which is important for appropriate management of patients and their families. Diagnosis of PRKAG2 cardiomyopathy necessitates electrophysiological monitoring due to the high incidence of conduction defects. *LAMP2* mutations cause childhood-onset cardiomyopathy with prevalent arrhythmias, often in the context of Danon disease (neurocognitive deficits and hepatic dysfunction), which has a very poor prognosis (65) and requires early triage to heart transplantation. *GLA* mutations cause Fabry disease (LVH with renal, eye, and skin manifestations), accounting for at least 1% of all cases of unexplained LVH and an even higher percentage of patients who present after 40 years of age (66-68). Recognition of Fabry cardiomyopathy has critical therapeutic implications, as early treatment with enzyme replacement therapy limits myocardial remodeling and maintains cardiac function (69-71).

RESTRICTIVE CARDIOMYOPATHY. Primary RCM is a very rare heart muscle disease that is diagnosed by functional, rather than anatomic findings. RCM causes increased myocardial stiffness that results in a rapid rise in ventricular pressure, despite only small increases in ventricular volumes (1,2). This functional definition complicates disease classification because restrictive physiology also occurs with HCM and DCM. Among 1,226 patients with familial HCM (688 families), 1.5% had phenotypes diagnostic of RCM (72). One-half of the probands with RCM had pathogenic mutations in either *MYH7* or *TNNI3*; all patients with an identified mutation either had marked myofibrillar disarray on biopsy or had relatives with an unequivocal diagnosis of HCM. Notably, restrictive physiology was associated with a higher symptom burden, lower exercise tolerance, and increased complications (72).

Additional mutations in *TNNI3* (71), as well as mutations in other sarcomere genes (73-75), and an unidentified gene at locus 10q23.3 (76) are also associated with RCM. Affected family members had HCM, DCM, or desmin-associated myopathy. Mutations in *MYPN*, which encodes the sarcomere-associated protein myopalladin, can also cause myofibrillar disarray and restrictive physiology (77). However the resulting clinical diagnoses associated with *MYPN* mutations include HCM, DCM, or RCM (77-80).

Two mutations associated with RCM have been modeled in mice. Mutant animals carrying a *TNNI3* missense mutation that was associated with human RCM and HCM, developed early diastolic dysfunction, small chamber size, and reduced LV systolic function, atypical manifestations of HCM (81). A *MYPN* mutation produced subtle manifestations of increased cardiac fibrosis, reduced LV chamber size, and minimal diastolic dysfunction (82).

In aggregate, these data illuminate the variable physiological and anatomic responses to sarcomere mutations, and implicate factors beyond a specific mutation in clinical manifestation. RCM does not appear to constitute a distinct genetic cardiomyopathy, but rather represents part of a phenotypic spectrum of HCM that occurs with limited hypertrophy and restrictive physiology.

DILATED CARDIOMYOPATHY. DCM is characterized by ventricular chamber enlargement and systolic dysfunction (1). LV mass is often greatly increased in DCM, but in contrast to HCM, LV wall thickness is normal. The histopathology of DCM is typically unrevealing of the underlying cause: myocytes are enlarged, with characteristics of cell demise, and myocardial fibrosis is increased, which are abnormalities that promote arrhythmias and HF. DCM is the commonest cause for heart transplantation. Approximately 50% of nonischemic DCM has no identifiable etiology (83), and in both familial and sporadic cases, genetic causes are increasingly identified.

Early population data using echocardiography estimated the prevalence of unexplained DCM at 1:2,500 (84). However, extrapolation of epidemiological data for DCM and HF suggests a much higher prevalence, possibly as high as 1:250 (85), an estimate that is aligned with the prevalence of likely pathogenic variants identified by next-generation sequencing (7,17).

DCM genes encode a heterogeneous group of molecules that participate in force generation, force transmission, sarcomere integrity, cytoskeletal and nuclear architecture, electrolyte homeostasis, mitochondrial function, and transcription (**Figure 2**, Online Table 3). Although most mutations are transmitted as dominant traits, a few exhibit recessive, *X*-linked, and matrilineal inheritance. Several issues contribute to the limited clinical recognition of familial DCM. First, the nonspecific manifestations of genetic DCM are frequently misattributed to other common and prevalent cardiovascular diseases. Second, DCM mutations exhibit age-dependent penetrance, and clinical expression may be delayed until after the fifth or sixth decade. Even with overt familial transmission, the penetrance of DCM mutations can be incomplete, an observation that indicates the potential for compensatory mechanisms, and modifying genetic and epigenetic factors. Finally, the substantial genetic heterogeneity of DCM has made comprehensive genetic testing technically difficult and costly. Until recently, most DCM diagnosis panels failed to screen all potential genes, resulting in disappointingly low sensitivity for detection of pathogenic mutations. Next-generation sequencing that can fully interrogate all DCM genes should substantially increase mutation detection in both familial and sporadic cases.

Selected DCM genes and functional consequences. *Mutations affecting sarcomere function.* Mutations in *TTN*, which encodes titin, a massive sarcomere protein, are the most prevalent genetic cause of DCM, accounting for ~15% to 20% of all DCM cases, including those with ambulatory and end-stage disease (42,86-88). Mutations that truncate titin (nonsense, frameshift, or splice-site and copy-number variants) cosegregate with familial DCM, providing overwhelming statistical evidence for pathogenicity (combined logarithm of odds score >11), and display nearly complete penetrance after 40 years of age (42). As clinical outcomes, including arrhythmic risk and progressive functional deficits, are worse in DCM patients with *TTN* truncating mutations than in patients with unknown etiologies, genetic diagnosis is expected to improve risk stratification and interventions (88).

Titin contains 4 major protein domains (Z-disk, I-band, A-band, M-band) that span one-half the length of the sarcomere (**Figure 3A**). There is considerable variation in the usage of exons that encode the I-band domains, whereas most other domains incorporate all exons (89). As a consequence, truncating mutations within I-band exons, if excluded from the mature transcript, will not cause disease, thereby accounting for the fact that ~3% of the general population with an I-band mutation do not have DCM (**Figures 3B and 3C**) (88). Genetic testing laboratories need to interpret *TTN* variants in light of exon usage (90) to appropriately discriminate between pathogenic and benign variants.

TTN sequence analyses have also revealed innumerable missense variants. Indeed, the Exome Sequencing Project identified on average 23 *TTN* missense variants per individual without cardiomyopathy (87), including approximately 1 rare *TTN* missense mutation (42). Functional analyses of cardiomyocytes derived from induced pluripotent human stem cells with a *TTN* missense mutation have demonstrated that at least some missense mutations can cause contractile dysfunction (89). Although the clinical significance of *TTN* missense variants remains unclear at present, this is an area of active investigation, and we expect at least some such mutations will be shown to cause disease, thereby further improving the clinical interpretation of these genetic variants.

RBM20 encodes ribonucleic acid binding motif 20 protein, a chaperone protein that binds messenger ribonucleic acid and regulates exon splicing to produce different gene isoforms. Among several cardiac targets, *RBM20* regulates splicing of titin (91). An

FIGURE 2 Dilated Cardiomyopathy Disease Genes
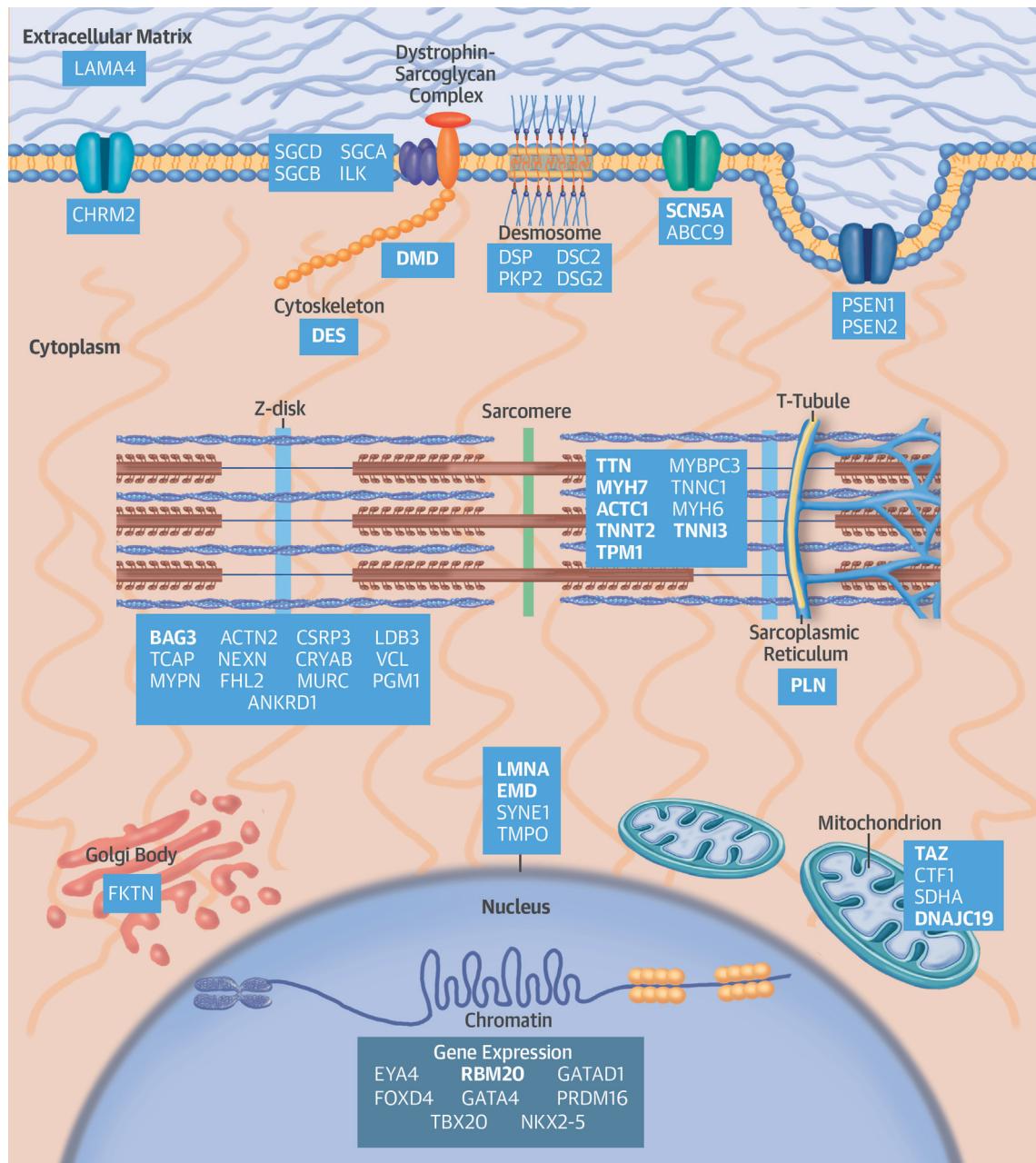
A schematic of definitive **(bold)** and posited dilated cardiomyopathy genes with the subcellular localization of the encoded proteins. Pathogenic genes encode proteins that participate in many diverse biological processes of cardiomyocytes.

RBM20 deletion in rats alters titin splicing and produces a DCM phenotype (92), providing strong evidence that both *RBM20* mutations and TTN abnormalities can cause cardiomyopathy. Multiple human *RBM20* mutations have now been identified, including a mutational hot spot in exon 9 (93-96).

Recurrent missense mutations in this exon alter an arginine-serine-rich region, which disrupts binding with other splicing factors and alters transcript processing, causing highly penetrant DCM (91).

Mutations affecting electrolyte homeostasis. Phospholamban mutations cause DCM by altering calcium

FIGURE 3   Titin Mutations in DCM

(A) Schematic representation and electron micrograph demonstrating the location of titin protein in the sarcomere. Titin molecules are composed of 4 protein domains, including the Z-disc (Z), I-band, A-band, and M-band, that span one-half the length of the sarcomere. (B) Bar graph demonstrating the percentage of identified *TTN* truncating variants in cohorts with a range of cardiovascular physiology. (C) Schematic demonstrating the location of *TTN* truncating variants with respect to protein domain. The percent spliced-in is a measure of exon usage in *TTN* messenger ribonucleic acid from different cardiac tissue samples and demonstrates reduced exon usage in the I-band, which would exclude the expression of most mutations located in this domain. In contrast, mutations causing dilated cardiomyopathy (DCM) reside in fully expressed exons. Adapted with permission from Roberts et al. (88). LV = left ventricular; OR = odds ratio.

homeostasis. Encoded by *PLN*, phospholamban regulates calcium uptake by the sarcoplasmic or endoplasmic reticulum calcium transporting ATPase (SERCA2a), functioning as a molecular brake on calcium cycling. Multiple distinct human mutations have been identified and confirmed with animal models (97-99). The Arg9Cys mutation in *PLN* is particularly severe, having been consistently identified with progressive HF requiring heart transplantation in early adult life in unrelated families (97,100,101). This mutation blunts β-adrenergic control of calcium cycling secondary to the reduced

FIGURE 3 Continued
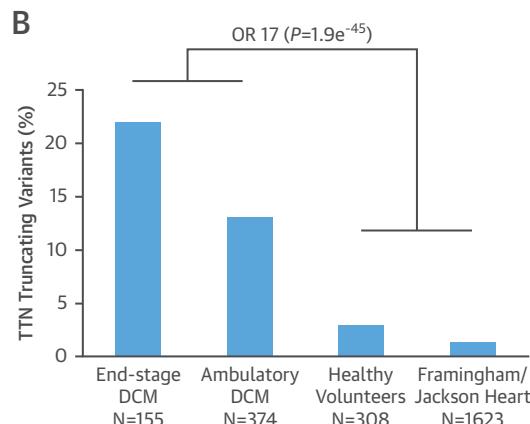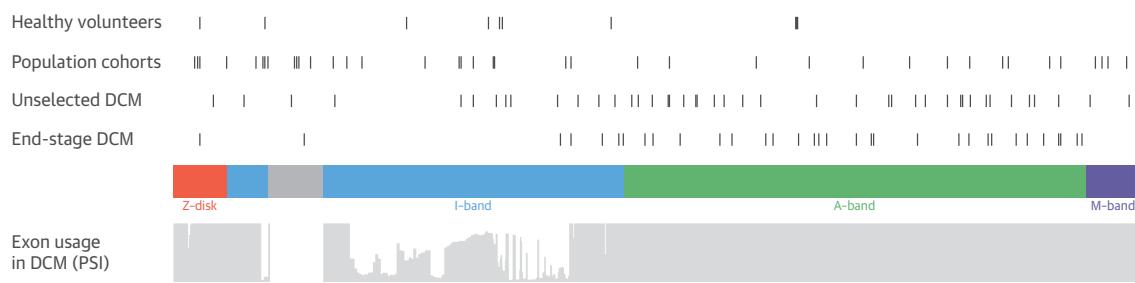
ability of protein kinase A to phosphorylate PLN (97,102,103). In turn, altered calcium kinetics leads to depressed contractility and DCM.

A single amino acid deletion in phospholamban (Arg14del) has been reported in association with mild and severe DCM phenotypes, perhaps indicating this as a genetic modifier rather than a pathogenic mutation (99,104). Consistent with this concept, PLN Arg14del has also been identified as a founder mutation that is >500 years old, and which constitutes 15% of index DCM cases in the Netherlands (105). Interestingly, this mutation also occurs in 12% of desmosome mutation-negative ARVC cases, all of which had a high burden of ventricular arrhythmias (105). The substantial clinical and pathological overlap observed in these subjects supports the growing finding that at least some forms of ARVC and DCM are not distinct clinical entities, but rather may constitute a condition sometimes labeled *arrhythmogenic cardiomyopathy*.

Some mutations in *SCN5A*, which encodes the sarcolemmal transmembrane cardiac voltage-gated sodium channel that functions in developing cardiac

action potentials, are implicated in DCM (106-108). *SCN5A* mutations also cause a high burden of arrhythmias, and constitute another example of arrhythmogenic cardiomyopathy (109). There are also many allelic variants in *SCN5A,* including those causing Brugada syndrome (110), idiopathic ventricular fibrillation (111), and familial atrial fibrillation (112). *Mutations affecting nonsarcomere structural proteins.* Multiple DCM genes cause both heart and skeletal muscle phenotypes (Online Table 3), including *LMNA*, which encodes lamin A/C, a ubiquitously expressed inner nuclear membrane protein that plays a role in maintenance of proper nuclear structure. Dominant *LMNA* mutations occur in approximately 6% of DCM cases, and are far more common in DCM with conduction system disease (113). Electrophysiological abnormalities (conduction system block and atrial fibrillation) often precede DCM that relentlessly progresses to HF (114,115). The severity of the associated skeletal myopathy is variable. Most *LMNA* mutations cause haploinsufficiency, and mouse models of these mutations demonstrate

inadequate response to mechanical strain, which may promote premature cardiomyocyte death (116).

DCM genotype-phenotype correlations. The marked genetic heterogeneity of DCM and variable penetrance of mutations has impeded the direct application of most genotypes to clinical management. A notable exception is *LMNA* mutations, which are highly predictive for progressive conduction disease and risk of sudden cardiac death (114,117,118). As initial manifestations of *LMNA* mutations are often subtle (e.g., first-degree atrioventricular block), clinical recognition of affected individuals at high risk for sudden death is difficult. This has prompted recommendations to restrict mutation carriers of any age from participation in competitive sports (117). Assessment for prophylactic implantable cardioverter-defibrillator placement to treat malignant ventricular arrhythmias should be considered for patients receiving a pacemaker for *LMNA*-associated conduction system disease, independent of ejection fraction (119,120). Likewise, atrial lead placement should be considered in *LMNA$^+$* individuals receiving an implantable cardioverter-defibrillator who do not otherwise meet criteria for dual-chamber pacemaker implantation.

OTHER GENETIC CARDIOMYOPATHIES. Mutations in additional genes have been identified as causing other cardiomyopathies, including LVNC (Online Table 4) (121,122), ARVC (Online Table 5) (123), mitochondrial cardiomyopathies (124), and the restrictive desminopathies (125). The interested reader is referred to the suggested reviews for further details.

GENETIC TESTING IN CLINICAL PRACTICE

Defining the precise genetic cause of a cardiomyopathy in patients can improve clinical management. The identification of a pathogenic mutation provides diagnostic certainty and eliminates ambiguities associated with phenotypic variation. Genetic data may also help to guide the use of emerging therapies that target the biophysical consequences associated with mutations (47). Genetic diagnosis enables cost-effective screening of first-degree family members and eliminates healthcare expenditures for relatives without pathogenic mutations, resulting in substantial health care cost savings (35).

Cardiomyopathy gene panels continue to evolve, but increasingly include comprehensive analyses of all genes implicated in HCM, DCM, ARVC, or LVNC. Commercially available genetic testing for cardiomyopathies (and other disorders) can be found at the GeneTests website (126). This site details available gene panels and performing laboratories with contact information and costs. Additional genetic testing information is found in the National Institutes of Health Genetic Testing Registry (127). Genetic testing costs vary considerably between commercial and academic laboratories, technology used, and numbers of genes analyzed. In the United States, patients are initially studied using large multigene cardiomyopathy panels that are sequenced by next-generation technologies (at commercial costs from $1,500 to $5,000). If negative, whole-exome or whole-genome sequencing analyses are considered. With the continued fall in technical and analysis costs, these broad-based platforms may become the preferred strategy for genetic testing of cardiomyopathies. The identification of a definitive mutation allows targeted analysis of first-degree relatives at substantially reduced costs (typically only a few hundred dollars).

INTERPRETING GENETIC TESTING. Genetic testing can yield 5 distinct results (**Table 2**) (4): 1) identification of a definitively pathogenic mutation, an outcome that confirms the diagnosis, establishes etiology, and identifies a target for familial screening; 2) identification of a probable pathogenic mutation, which is a potentially useful result that supports the clinical diagnosis, but additional evidence, such as familial cosegregation or confirmatory data in unrelated affected patients, is necessary to establish causality; 3) identification of a variant of unknown significance, which is a result that does not distinguish whether a variant is causative or represents a rare polymorphism unrelated to the disease. Familial segregation of a identification of a variant of unknown significance can support research aimed at identifying new disease genes or can be used to characterize a newly identified variant as likely pathogenic; 4) identification of benign variants, which is a result that identifies a polymorphism that is not disease associated and occurs in the general population; and 5) no variant is identified. Scenarios 4 and 5 can indicate that an unknown gene, or a gene not included in the analyses causes the disease, or that the condition is not genetic. In these instances, continued clinical surveillance, rather than genetic testing of first-degree relatives, is necessary.

Increasingly, genetic testing reports provide an assessment of the pathogenicity of all identified variants. A clinician trained in medical genetics or a clinical genetic counselor should always review the interpretation of the results (128). Clinical genetics is a rapidly advancing field, and proper communication of genetic testing results requires knowledge of advances in testing, emerging data on newly identified

variants and clinical correlations, and an understanding of the complex allelic heterogeneity that characterizes inherited cardiomyopathies. Complicated scenarios can arise, including the discovery of multiple variants that may influence disease. Furthermore, given the age of onset for many genetic cardiomyopathies, a major point of discussion is reproductive counseling. The limits of cardiovascular disease training, the rapidly advancing field of clinical genetics, and the constraints on physician time often require the involvement of a clinical genetic counselor to fully communicate these results.

THE GENOTYPE-POSITIVE PHENOTYPE-NEGATIVE INDIVIDUAL. Genetic testing of cardiomyopathies has uncovered a new class of patients: mutation-positive/phenotype-negative individuals. Often these individuals are younger than the typical age at which clinical disease is recognized. This group poses many uncertainties, including the periodicity for serial clinical evaluations, whether participation or exclusion from intensive athletics is warranted, and whether this status and family history of sudden cardiac death warrants prophylactic interventions. The complexities of managing genotype-positive phenotype-negative individuals reflect: 1) a paucity of outcome data; 2) incomplete knowledge about the molecular mechanisms that link gene mutation and disease; and 3) variable disease penetrance, which may indicate uncharacterized compensatory mechanisms that modify disease. Although consensus documents provide opinions from experts in genetic cardiomyopathies, this is an area of active research that may influence recommendations (**Central Illustration**) (10,129-131).

Longitudinal study of genotype-positive phenotype-negative individuals provides the unique opportunity to study the natural history of disease, including phenotype conversion. Recent advances in the development of human cardiomyocytes derived from induced pluripotent stem cells with cardiomyopathy mutations are expected to provide a more comprehensive understanding of critical molecular signals that may not be recapitulated by mouse models (132). The study of human subjects, coupled with animal and cell models, should help elucidate the roles of genetic modifiers and epigenetic changes that likely contribute to the variability in phenotype (133).

Detailed analyses of genotype-positive phenotype-negative subjects have also identified functional abnormalities that precede cardiac remodeling (134-136). These subtle manifestations raise a critical question: Can development of cardiomyopathy be delayed or prevented by treatment of those with preclinical disease? Disruption of key molecular pathways in animal models of HCM before disease onset has been shown to blunt and even reverse hypertrophic remodeling (137,138). Although results from small clinical trials have been promising (139,140), the recently published INHERIT (INHibition of the renin angiotensin system in hypertrophic cardiomyopathy and the Effect on hypertrophy—a Randomised Intervention Trial with losartan) trial failed to show a reduction in LVH with losartan treatment in HCM subjects with established disease (141), suggesting that strategies that capitalize on genetic testing to identify early disease in mutation-positive patients may be warranted. The currently enrolling VANISH (Valsartan for Attenuating Disease Evolution In Early Sarcomeric HCM) trial (NCT01912534) seeks to assess whether treatment with valsartan early in HCM can slow disease progression.

SUMMARY AND RECOMMENDATIONS. The use of clinical genetic testing is rapidly evolving in conjunction with advances in sequencing technology, new discoveries about the genetic basis of disease, and reduced costs, leading to more widespread coverage by medical insurance. As such, the number of cardiomyopathy patients who should have genetic testing is rapidly growing. Although the decision to perform genetic testing should be individualized, when applied appropriately, genetic testing assists with diagnosis and guides the clinical management of the patient and family members (**Central Illustration**).

Genetic testing can remove the ambiguity of clinical diagnosis (i.e., mild ventricular remodeling in a competitive athlete), and influence clinical screening and management of family members. This is particularly important with children and adolescents who may not yet have manifested clinical features of disease, but who may be at risk for sudden death. Contemporary guidelines recommend targeted genetic testing within families with a definitive pathogenic mutation. Genotype-positive phenotype-negative individuals require continued clinical screening (**Central Illustration**), whereas relatives without the familial mutation can be reassured and require no further screening. Although first-degree relatives with overt disease may not directly benefit from genetic testing, such results can inform the clinical interpretation of variants with less certain pathogenicity. For the individual with a definitive clinical diagnosis of cardiomyopathy and no relatives at risk for disease, genetic testing can still contribute to increasing knowledge of how genotype influences phenotype and outcomes.

CENTRAL ILLUSTRATION   Genetics of Inherited Cardiomyopathies

A   **Clinical screening for relatives of cardiomyopathy patients**

- Personal and family history
- Physical examination
- Electrocardiogram
- Echocardiogram
- For hypertrophic cardiomyopathy phenotype:
  - Holter monitor
  - Exercise treadmill test

Phenotype	Interval screening schedule
Hypertrophic cardiomyopathy	• Optional during early childhood; prudent in: - families with sudden death - early adverse events - children involved in competitive athletics - symptoms or clinical suspicion of early disease manifestations • Yearly during adolescence • Every 5 years or with symptom onset in adults
Dilated cardiomyopathy	• Every 1–2 years in childhood and adolescence • Every 3–5 years or with symptom onset in adults

B   **Cascade genetic screening in families**

Proband
Sequence analysis of:
- Disease-specific gene panel
- Whole exome/genome

At-risk relatives
Sequence analysis of the pathogenic or likely pathogenic mutation identified in the proband

Genetic screen of proband with suspected genetic cardiomyopathy

Mutation* is **not** identified in proband → Initial clinical screening for at-risk relatives → Interval screening for at-risk relatives

Mutation* is identified in proband → Initial clinical screening for at-risk relatives → Targeted mutation testing for at-risk relatives → Mutation-positive → Clinical screening / Mutation-negative

Cardiomyopathy identified: Management

Cardiomyopathy **not** identified: Interval clinical screening

No further screening is required

*Mutations include pathogenic or likely pathogenic variations

Burke, M.A. et al. J Am Coll Cardiol. 2016;68(25):2871–86.

(A) Clinical or **(B)** cascade genetic screening strategies for familial cardiomyopathies. Initial clinical and genetic screening, as well as interval follow-up, is recommended as per guidelines (10,143,144).

FUTURE DIRECTIONS

The last quarter-century has provided remarkable progress in cardiomyopathy genetics, but there is much yet to learn. Continued application of technical advances in genomic sequencing is expected to further unlock the genetic basis of these enigmatic disorders. In addition to identifying new mutations that alter protein sequences, these technologies can identify structural genomic changes and variations in regulatory elements that can alter gene dosage without affecting protein sequences. Additional research will improve our understanding of the complex and longitudinal molecular changes that lead from gene mutation to clinical expression.

Future strategies may directly target the mutant allele itself. Pre-clinical assessment of this approach in an HCM mouse model successfully employed

adenoviral constructs to silence the mutant, but not the wild-type allele, resulting in complete abrogation of the disease phenotype for many months (142). Further development of similar approaches raises the possibility that someday genetic cardiomyopathies will not only be treated, but also be cured.

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APPENDIX For supplemental Methods and References sections as well as supplemental tables, please see the online version of this article.