



REVIEW

Inherited Cardiomyopathies

Molecular Genetics and Clinical Genetic Testing in the Postgenomic Era

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Inherited cardiomyopathies include hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, left ventricular noncompaction, and restrictive cardiomyopathy. These diseases have a substantial genetic component and predispose to sudden cardiac death, which provides a high incentive to identify and sequence disease genes in affected individuals to identify pathogenic variants. Clinical genetic testing, which is now widely available, can be a powerful tool for identifying presymptomatic individuals. However, locus and allelic heterogeneity are the rule, as are clinical variability and reduced penetrance of disease in carriers of pathogenic variants. These factors, combined with genetic and phenotypic overlap between different cardiomyopathies, have made clinical genetic testing a lengthy and costly process. Next-generation sequencing technologies have removed many limitations such that comprehensive testing is now feasible, shortening diagnostic odysseys for clinically complex cases. Remaining challenges include the incomplete understanding of the spectrum of benign and pathogenic variants in the cardiomyopathy genes, which is a source of inconclusive results. This review provides an overview of inherited cardiomyopathies with a focus on their genetic etiology and diagnostic testing in the postgenomic era. (*J Mol Diagn* 2013, 15: 158–170; <http://dx.doi.org/10.1016/j.jmoldx.2012.09.002>)

Inherited cardiomyopathies are a group of cardiovascular disorders classified based on ventricular morphology and function and include hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), left ventricular noncompaction (LVNC), and restrictive cardiomyopathy (RCM).¹ Of these, HCM is the most common, with an estimated prevalence of 1 in 500 individuals. Common complications can include heart failure and sudden cardiac death (SCD), and undiagnosed disease (particularly in HCM and ARVC)

accounts for a significant proportion of SCD in young adults and athletes.²

Since the discovery that pathogenic variants in the sarcomere genes cause HCM,³ many advancements have been made to define the genetic etiology of inherited cardiomyopathies. The increased risk of SCD in individuals with disease encouraged interest in clinical genetic testing, which began approximately a decade ago and is now widely

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available through genetic testing laboratories in the United States and worldwide (<http://www.genetests.org> and <http://www.ncbi.nlm.nih.gov/gtr>). However, the high degree of locus and allelic heterogeneity that is characteristic of all cardiomyopathies necessitates sequence analysis of the entire coding region of multiple genes, which has been a costly and lengthy process using traditional technologies. Growing genetic and phenotypic overlap among different cardiomyopathies adds further complexity, often resulting in stepwise testing of multiple disease-specific gene panels when the diagnosis is not entirely clear. Next-generation sequencing (NGS) technologies have removed these barriers and enabled testing of a large number of genes simultaneously. NGS-based test panels are now offered by a growing number of diagnostic laboratories worldwide, which is beginning to change the landscape of medical genetic testing. However, an unwelcome effect of this unprecedented ability to sequence any gene is an increased likelihood of detecting variants of unclear clinical significance (VUSs). This effect necessitates a stringent review of the evidence supporting claims of disease association as variants in insufficiently studied genes can be difficult to interpret. For example, variants in >50 genes have been reported to be causative for various inherited cardiomyopathies, but a detailed review and assignment of a confidence level shows that only half of them meet the criteria to be considered a definitive disease gene (Table 1).

Practice guidelines and expert opinions on clinical management and genetic testing for inherited cardiomyopathies recommend taking a detailed family history that includes at least three generations, clinically screening at-risk family members, counseling patients on a possible inherited cause, and considering genetic testing of the most clearly affected person in the family.^{4,5} However, current guidelines recommend comprehensive or targeted genetic testing for only a small number of genes.⁴ This is in contrast to the increasing use of large gene panels in clinical practice.

For inherited cardiomyopathies, therapeutic options are limited, and, thus, the clinical utility of genetic testing largely lies in the ability to establish or confirm the disease etiology in the proband (when a definitively pathogenic variant is identified). Subsequent testing of at-risk family members can remove the disease risk (when negative) or identify those in need of clinical monitoring or intervention to decrease the risk of morbidity or mortality (when positive). However, because genetic testing for inherited cardiomyopathies is a relatively young discipline, the spectrum of pathogenic variants present in the population is incompletely characterized, even in well-established disease genes. This leaves a high probability of detecting a novel sequence VUS, which can create substantial emotional difficulties for patients.

This review summarizes the clinical features of inherited cardiomyopathies, current knowledge on the genetic etiologies, and clinical genetic testing in the postgenomic era.

Hypertrophic Cardiomyopathy

Clinical Description and Disease Mechanism

HCM is characterized by left ventricular hypertrophy (LVH) in the absence of an underlying systemic condition or other cardiac disease, such as valvular heart disease or hypertension. With an estimated prevalence of 1 in 500 in the general population,^{6,7} HCM is the most common inherited heart condition and the leading cause of sudden nontraumatic death in young adults and competitive athletes in the United States.⁸ Although the age at onset of HCM can range from infancy to old age, manifestations usually do not appear before adolescence in carriers of a pathogenic variant.⁸ HCM is primarily inherited in an autosomal dominant pattern, although reduced penetrance and clinical variability are common. Clinical manifestations range from being completely asymptomatic to progressive heart failure and SCD caused by mechanical or electrical defects. HCM is traditionally diagnosed using cardiac imaging modalities, such as echocardiography and cardiac magnetic resonance imaging, and often presents as asymmetrical septal hypertrophy.⁹ A complete list of features associated with classic HCM is shown in Supplemental Table S1. Histopathologic hallmarks include myocyte hypertrophy and disarray and increased myocardial fibrosis (for a comparison of normal cardiac histology and a heart with HCM showing disarray and fibrosis see the article by Cirino and Ho⁹). These lesions ultimately lead to impaired diastolic function.¹⁰ In a small proportion of patients (~5% to 10%), cardiac function worsens over time, leading to progressive LV dilatation, heart failure, and, ultimately, burnt-out HCM, which is morphologically similar to DCM.¹¹

HCM is frequently described as a disease of the sarcomere, and pathogenic variants have been detected in almost all sarcomere proteins.³ Molecular mechanisms include increased actin-activated ATPase activity, disruption of actin-myosin interaction and force generation, and altered intracellular calcium signaling in cardiomyocytes.¹² In addition, some data suggest that LVH can be caused by perturbations of transforming growth factor β and CaMKII Mef2 signaling pathways.¹³

The differential diagnosis of HCM includes several syndromes that typically manifest with multiorgan involvement but that can also present with isolated or predominant LVH. These syndromes include metabolic (storage) cardiomyopathies, such as Danon disease and Wolff-Parkinson-White syndrome,¹⁴ and the lysosomal storage disorder Fabry disease.¹⁵ LVH in these conditions is not accompanied by myocyte disarray or fibrosis but by a characteristic accumulation of glycogen or glycosphingolipids in cellular vacuoles.¹⁴ LVH is also a part of the phenotypic spectrum of Noonan syndrome¹⁶ and Friedreich ataxia.¹⁷

Genetic Etiology and Clinical Testing

Pathogenic variants for HCM have been described in eight genes encoding sarcomere proteins, with most (~80%) present

Table 1 Cardiomyopathy Genes and Associated Clinical Features

Gene	Evidence level*	Spectrum of clinical features						Inheritance	Location/role [‡]
		HCM	DCM	ARVC	LVNC	RCM	Other cardiovascular/muscular [†]		
<i>ABCC9</i>	3		X					AD	Potassium channel [§]
<i>ACTC1</i>	1	X [¶]	X		X	X		AD	Sarcomere
<i>ACTN2</i>	2	X [¶]	X					AD	Z-disk
<i>ANKRD1</i>	3	X	X					IU	Z-disk
<i>BAG3</i>	2	X	X [¶]			X	Myofibrillar myopathy	AD	Z-disk
<i>CASQ2</i>	1				X		CPVT [¶]	AR	Sarcoplasmic reticulum
<i>CAV3</i>	3	X	X				Myopathies, long QT, hyperCKemia	AD, AR, IU	Plasma membrane
<i>CRYAB</i>	1		X				Myofibrillar myopathy [¶]	AD, AR, IU	Chaperone
<i>CSRP3</i>	2	X [¶]	X				Myopathy	AD	Z-disk
<i>CTF1</i>	3		X					IU	Cytokine [§]
<i>DES</i>	2		X [¶]	X		X	Myofibrillar myopathy	AD	Intermediate filament
<i>DSC2</i>	1		X	X [¶]				AD	Desmosome
<i>DSG2</i>	1		X	X [¶]				AD	Desmosome
<i>DSP</i>	1		X	X [¶]			Carvajal syndrome [¶]	AD, AR	Desmosome
<i>DTNA</i>	2				X [¶]			AD	Dystrophin-associated protein complex
<i>EMD</i>	1		X				Emery-Dreifuss muscular dystrophy [¶]	XL	Nuclear membrane
<i>FHL2</i>	3		X					IU	Z-disk
<i>GATAD1</i>	2		X [¶]					AR	Unknown [§]
<i>GLA</i>	1	X					Fabry disease [¶]	XL	Lysosome [§]
<i>JUP</i>	2			X [¶]			Naxos disease [¶]	AD, AR	Desmosome
<i>LAMA4</i>	3		X					IU	Basement membrane
<i>LAMP2</i>	1	X	X				Danon disease [¶]	XL	Lysosome [§]
<i>LDB3</i>	2	X	X [¶]		X		Myofibrillar myopathy	AD	Z-disk
<i>LMNA</i>	1		X [¶]		X		Myopathies, muscular and lipodystrophies	AD	Nuclear membrane
<i>MYBPC3</i>	1	X [¶]	X		X			AD	Sarcomere
<i>MYH6</i>	3	X	X				CHD	AD	Sarcomere
<i>MYH7</i>	1	X [¶]	X		X	X	Myopathies	AD	Sarcomere
<i>MYL2</i>	1	X [¶]						AD	Sarcomere
<i>MYL3</i>	1	X [¶]						AD	Sarcomere
<i>MYLK2</i>	3	X						IU	Kinase [§]
<i>MYOZ2</i>	2	X [¶]						AD	Z-disk
<i>NEBL</i>	3		X				Endocardial fibroelastosis	IU	Z-disk
<i>NEXN</i>	3	X	X					AD	Z-disk
<i>PKP2</i>	1		X	X [¶]				AD	Desmosome
<i>PLN</i>	1	X	X [¶]					AD	Sarcoplasmic reticulum
<i>PRKAG2</i>	1	X					WPW, glycogen storage disease [¶]	AD	Kinase [§]
<i>PSEN2</i>	3		X					IU	Transmembrane protein [§]
<i>RBM20</i>	1		X [¶]					AD	RNA-binding protein [§]
<i>RYR2</i>	1	X		X			CPVT [¶]	AD	Sarcoplasmic reticulum
<i>SCN5A</i>	1		X [¶]				Brugada syndrome, [¶] long QT [¶]	AD	Sodium channel
<i>SGCD</i>	1		X				Limb-girdle muscular dystrophy [¶]	AR, AD, IU	Dystrophin-associated protein complex
<i>TAZ</i>	1		X		X		Barth syndrome [¶]	XL	Mitochondrion [§]
<i>TCAP</i>	2	X	X				Limb-girdle muscular dystrophy [¶]	AR, AD, IU	Z-disk
<i>TMEM43</i>	1			X [¶]				AD	Transmembrane protein [§]
<i>TMPO</i>	3		X					IU	Nuclear membrane
<i>TNNC1</i>	2	X	X [¶]					AD	Sarcomere
<i>TNNI3</i>	1	X [¶]	X			X		AD	Sarcomere
<i>TNNT2</i>	1	X [¶]	X		X	X		AD	Sarcomere
<i>TPM1</i>	1	X [¶]	X					AD	Sarcomere
<i>TTN</i>	1	X	X [¶]	X			Myopathies	AD	Sarcomere
<i>TTR</i>	1	X					Amyloidosis [¶]	AD	Transport protein [§]
<i>VCL</i>	3	X	X [¶]		X			AD	Z-disk

in the *MYH7* and *MYBPC3* genes (Table 1).^{1,3} As is typical for structural proteins, most sarcomere variants are believed to act in a dominant negative manner (ie, by adversely affecting the normal gene product). Loss-of-function variants leading to haploinsufficiency occur less frequently but are prevalent in the *MYBPC3* gene.¹⁸ Collectively, sarcomere variants are identified in up to 60% of patients with HCM who also have a family history and in ~40% of patients with sporadic HCM.¹⁹ Storage cardiomyopathies masquerading as HCM are caused by mutations in *LAMP2* (Danon disease), *PRKAG2* (Wolff-Parkinson-White syndrome), and *GLA* (Fabry disease).

Recently, the spectrum of genes associated with HCM has been expanded to nonsarcomeric genes and includes genes encoding Z-disk proteins and proteins located in the sarcoplasmic reticulum and plasma membrane (Table 1). However, variants in these genes are rare, and studies providing evidence of a role in disease are limited. Some genes are associated by strong genetic evidence, such as segregation with disease or *in vivo* functional data (eg, *CSRP3*),²⁰ but many genes (eg, *MYH6*, *MYLK2*, and *TCAP*) are supported only by the presence of variants in affected individuals, absence from controls, and perhaps computational assessments. These genes are better described as candidate genes (Table 1). Although almost 1000 variants for HCM have been identified to date,¹⁹ most are private and can, therefore, be detected only through comprehensive genetic testing. A small number of recurring variants are present at higher population frequencies, the most common of which is a 25-bp deletion in intron 32 of the *MYBPC3* gene, which is prevalent in Southeast Asian populations (~4%), accounting for an increased risk of heart failure with an odds ratio of ~7.²¹

With few exceptions, genotype-phenotype correlations for HCM are incompletely defined. Those that have been established are broad correlations at the gene level. For example, some, but not all, *TNNT2* mutations are associated with only minor hypertrophy but an appreciable risk of arrhythmia.²² *MYH7* variants generally seem to lead to significant LVH by the second decade of life and are thought to be associated with an increased risk of heart failure and SCD.²³ In contrast, pathogenic variants in *MYBPC3* are believed to be associated with a later age at onset,²⁴ although they have also been identified in a significant proportion of patients with childhood-onset LVH.²⁵ Most *MYBPC3* variants in these pediatric patients were missense changes, which contrasts with the high prevalence

of loss-of-function variants identified in the adult HCM population and suggests that missense variants may have more severe functional consequences.²⁵

Recent clinical guidelines for HCM recommend comprehensive testing for five HCM genes (*MYBPC3*, *MYH7*, *TNNI3*, *TNNT2*, and *TPM1*).⁴ Sequencing panels including, but not limited to, these genes are offered by several laboratories in the United States and worldwide (<http://www.genetests.org> and <http://www.ncbi.nlm.nih.gov/gtr>).

Currently, genetic testing for HCM is primarily used to identify families with a detectable genetic cause of disease and to screen at-risk family members. Testing can also help rule out nongenetic conditions, such as athlete's heart, although only when a pathogenic variant is identified.^{19,26} Owing to the lack of clear genotype-phenotype correlations, the use of genetic test results in guiding clinical management is limited. A notable exception is enzyme replacement therapy for storage disorders that can present as isolated LVH.²⁷

An emerging area is the use of genotype data to guide therapeutic decisions in preclinical individuals. Animal studies suggest that calcium channel blockers, such as diltiazem, may delay the clinical progression of disease, and clinical trials are currently under way.²⁸ In addition, animal studies have shown evidence of a link between sarcomeric HCM and increased transforming growth factor β signaling. An anti-transforming growth factor β antibody and losartan (an angiotensin II receptor type 1 antagonist) prevented cardiac fibrosis and hypertrophy in sarcomere mutation-positive mice, which may open additional therapeutic avenues.¹³

Dilated Cardiomyopathy

Clinical Description and Disease Mechanism

DCM is defined by LV dilatation and systolic dysfunction (a reduction in myocardial force generation characterized by an ejection fraction of <50%) and is the most common indication for cardiac transplantation in the United States, as reviewed by Hershberger et al.²⁹ The spectrum of clinical manifestations includes heart failure, thromboembolism, and SCD. DCM can also be an end-stage presentation of other diseases or environmental exposures, including myocarditis and alcohol abuse. Idiopathic DCM, which is diagnosed when known systemic or environmental triggers have been excluded, includes genetic DCM. The estimated prevalence

*Genes were classified into three levels with increasing evidence supporting a role in disease based on the following criteria. All reported variants were absent from a large number of controls. Level 1: multiple studies, variants, and families reported and segregation with disease established. Level 2: single or few studies, variants, and families reported and limited segregation observed. Level 3: single or few studies, variants, and families reported and segregation not established.

[†]Additional cardiovascular or muscular features/conditions with or without cardiomyopathy.[‡]Location or role of gene depicted in Figure 1.

[§]Location not depicted in Figure 1. Additional references used to arrive at the evidence levels are available on request.

[¶]Disease association serving as the primary basis for evidence level.

^{||}Cardiomyopathy seen as part of a larger disease spectrum (disease noted).

AD, autosomal dominant; AR, autosomal recessive; CPVT, catecholaminergic polymorphic ventricular tachycardia; CHD, congenital heart disease; IU, inheritance unknown; WPW, Wolff-Parkinson-White syndrome; X, disease/gene association; XL, X-linked.

of idiopathic DCM is 1 in 2500 individuals, although this may be an underestimate, and the percentage of idiopathic DCM cases that have a genetic etiology is estimated to be 30% to 50% based on the presence of a family history.²⁹ The age at onset can vary from childhood to late adulthood, although most patients are diagnosed between 20 and 50 years of age.³⁰ In contrast to HCM, myocyte disarray is absent, although myocyte hypertrophy and increased fibrosis may be observed.³¹ Unlike HCM, contractile dysfunction and ventricular remodeling in DCM are typically progressive. Although penetrance is age dependent and incomplete, earliest overt DCM is characterized by isolated LV dilatation and/or mild LV systolic dysfunction. The histopathologic features of this stage have not been well described. Over a varying amount of time, LV remodeling continues and may progress to severe dilatation and systolic dysfunction with nonspecific histopathologic features of myocyte hypertrophy and interstitial fibrosis. Ventricular remodeling may culminate in fatal end-stage heart failure, arrhythmia, or a cardioembolic event (eg, stroke). Nevertheless, robust natural history studies do not exist to inform the rate or likelihood of progression from mild to severe disease.

DCM can also present with muscular involvement and may be the presenting or primary clinical feature of several multisystemic conditions, including Emery-Dreifuss muscular dystrophy, Barth syndrome, myofibrillar myopathy, and Duchenne's muscular dystrophy. Furthermore, the spectrum of genetic DCM has recently been expanded to include peripartum cardiomyopathy based on familial co-occurrences of peripartum cardiomyopathy and DCM.³²

Compared with HCM, which is mainly a disease of the sarcomere, DCM shows a considerably higher degree of locus heterogeneity with a steadily growing number of genes implicated (currently ~40) (Table 1). These genes are part of a functional continuum that includes the sarcomere, Z-disk, nuclear lamina proteins, intermediate filaments, and the dystrophin-associated glycoprotein complex (Figure 1).³³ Molecular mechanisms include diminished force generation and transmission, alterations of energy production and regulation, and intracellular calcium defects, as reviewed by Watkins et al.³⁴ As is true for all cardiomyopathies, DCM is most commonly inherited in an autosomal dominant pattern, although X-linked and autosomal recessive inheritances have been described.

Genetic Etiology and Clinical Testing

Owing to significant locus and allelic heterogeneity, genetic testing for DCM is only now becoming more widely used in clinical practice, and, therefore, the variant spectrum and detection rates are less defined than are those for HCM. It is estimated that pathogenic variants can be identified in 17% to 30% of individuals with DCM when up to 20 genes are sequenced. Unlike for HCM, most DCM genes contribute only a small percentage of all pathogenic variants.^{35–38} Clear genotype-phenotype correlations are rare. Exceptions

include variants in the *LMNA* and *SCN5A* genes, which are typically associated with DCM and conduction system disease.^{39,40} The electrophysiologic manifestations usually appear before the onset of DCM and may be the only cardiac features.⁴¹ Recent evidence suggests that desmosomal genes, traditionally known to cause ARVC, may also be involved in the etiology of DCM,^{42,43} and new genes for DCM are continually being discovered, with recent additions including the gene encoding BCL2-associated athanogene 3 (*BAG3*), *RBM20*, and titin (*TTN*) (Table 1). *TTN* may contribute up to 25% of familial and 18% of sporadic DCM cases,⁴⁴ making it by far the most commonly mutated gene in DCM and potentially elevating the detection rate of genetic testing panels to that of HCM.

Current guidelines have not yet incorporated these recent discoveries and recommend comprehensive testing for the *LMNA* and *SCN5A* genes in patients with DCM who also have significant cardiac conduction disease and/or a family history of premature SCD.⁴ Genetic testing can allow for informed family evaluations if a pathogenic variant is identified in the proband. In addition, there is evidence that patients with asymptomatic systolic dysfunction benefit from pharmacologic treatment.⁴⁵

Arrhythmogenic Right Ventricular Cardiomyopathy

Clinical Description and Disease Mechanism

ARVC is defined by myocyte loss and fibrofatty infiltration of the myocardium is associated with an increased susceptibility to arrhythmias and sudden death and accounts for a significant portion of sudden deaths in athletes and young adults.⁴⁶ Initially thought to affect only the right ventricle, LV involvement is now becoming increasingly recognized.⁴⁷ The prevalence of ARVC is estimated to be 1 in 2000 to 5000 individuals, with 30% to 50% of cases being familial.⁴⁸ ARVC is typically inherited in an autosomal dominant pattern with reduced penetrance and variable expressivity and affects men more frequently than women.⁴⁹ Often appearing in young adults, ≥80% of cases are diagnosed before 40 years of age. Beginning in 1994, an international task force established criteria for a clinical diagnosis of ARVC based on electrocardiographic findings (repolarization, depolarization, or conduction abnormalities), the presence of arrhythmias, structural defects, histopathologic features, and familial features. These criteria, revised in 2010, are divided into six categories with major and minor features, and an individual is considered to meet the task force criteria for ARVC when two major, one major and two minor, or four minor criteria are fulfilled.⁵⁰ A definitive diagnosis can be challenging, in part owing to phenotypic overlap with other genetic and acquired cardiomyopathies.

ARVC is commonly described as a disease of the desmosome, a multiprotein complex that forms cell-to-cell junctions and links intermediate filaments of adjacent cells,

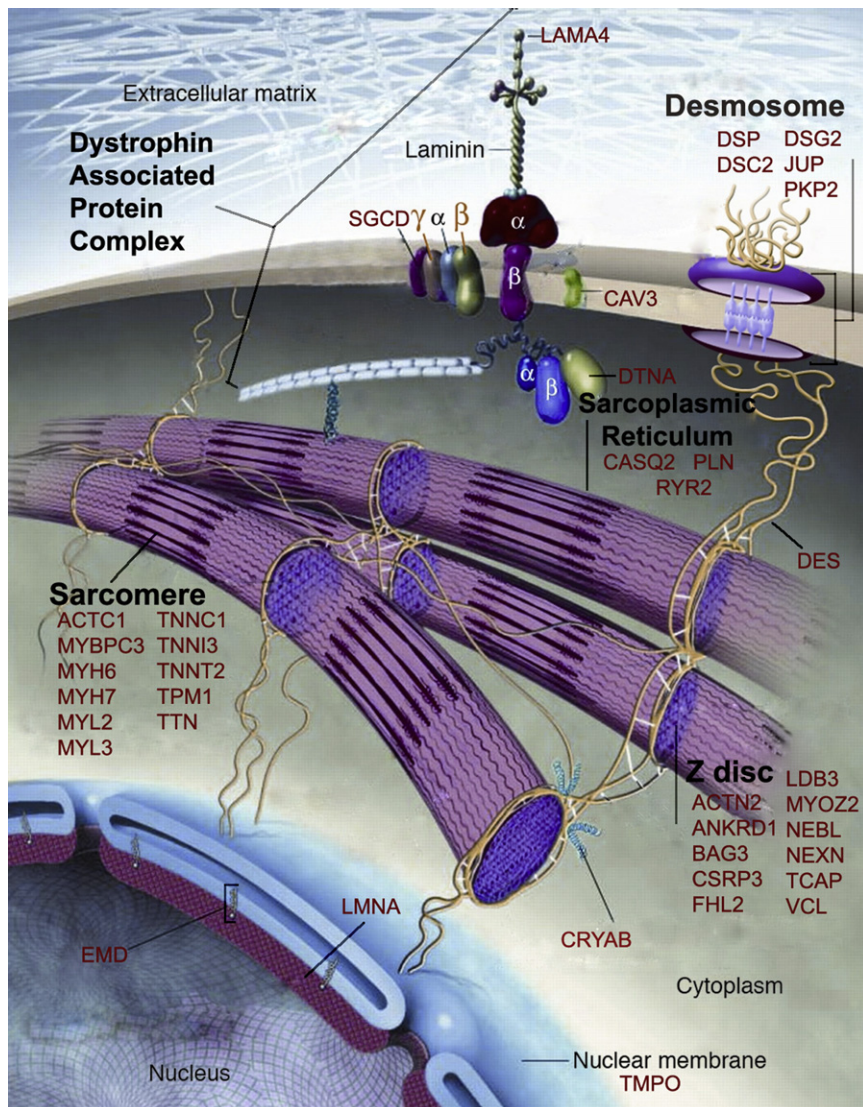


Figure 1 Cellular locations of proteins involved in inherited cardiomyopathies. Structural components of the myocyte that are impacted by cardiomyopathy include the sarcomere, Z-disk, nuclear lamina proteins, intermediate filaments, the desmosome, and the dystrophin-associated glycoprotein complex. Genes linked to cardiomyopathy and the locations of their protein products are indicated. Adapted with permission from the American Society for Clinical Investigation.³³

thereby establishing a functional intercellular network. Desmosomes are especially prevalent in tissues that are subjected to mechanical stress, such as cardiac muscle or skin, which explains why the phenotypic spectrum of ARVC encompasses cardiac and skin manifestations (see *Genetic Etiology and Clinical Testing* below). Molecular mechanisms of ARVC include impaired cell-cell adhesion and defective transmission of the contractile force.⁵¹ Recent evidence suggests that fibroadiposis is associated with impaired WNT signaling, which leads to a redirection of myocyte fate to adipocyte fate.⁵¹

Genetic Etiology and Clinical Testing

Most pathogenic ARVC variants are present in five genes encoding desmosomal proteins [plakoglobin (*JUP*), desmoplakin (*DSP*), desmocollin-2 (*DSC2*), desmoglein-2 (*DSG2*), and plakophilin-2 (*PKP2*)].⁵² In addition, homozygous or compound heterozygous *DSP* and *JUP* variants have been

described in patients with cardiomyopathy (DCM or ARVC), woolly/kinky hair, and palmoplantar hyperkeratosis (Naxos and Carvajal syndromes⁵³).

A few nondesmosomal ARVC genes have been described, including the transmembrane protein 43 (*TMEM43*). Although the evidence linking this gene to ARVC is strong, only one pathogenic variant has been described.⁵⁴ In a recent study showing some evidence that variants in the *TTN* gene may play a role in the etiology of ARVC, several missense variants were identified and one segregated with disease in multiple affected individuals.⁵⁵ Other proposed, although less convincing, ARVC genes include *TGF β 3*⁵⁶ and *RYR2*, a gene associated with catecholaminergic polymorphic ventricular tachycardia.⁵⁷

Analysis of the coding sequence of all desmosomal genes will identify a variant in up to 50% of individuals with ARVC, with ~40% of cases carrying a pathogenic variant in the *PKP2* gene.⁵² Current guidelines recommend targeted, variant-specific testing for family members when

a pathogenic variant has been identified in a proband but rank the utility of comprehensive screening of the *DSC2*, *DSG2*, *DSP*, *JUP*, and *TMEM43* genes lower than the utility of screening for HCM.⁴

Left Ventricular Noncompaction

Clinical Description and Disease Mechanism

Isolated LVNC is characterized by a heavily trabeculated or spongy appearance of the LV myocardium. An arrest of myocardial compaction during the first trimester of embryonic development is widely believed to be a cause although others have proposed that it can be an acquired process based on case observations of LVNC after previous normal echocardiographic findings (reviewed by Oechslin and Jenni⁵⁸). A recent literature survey suggests that LVNC is frequently associated with mitochondrial disorders,⁵⁹ followed by Barth syndrome, an X-linked condition characterized by early-onset cardiomyopathy (usually dilated, sometimes LVNC), neutropenia, muscle weakness, and growth delay.

Although the true prevalence of LVNC is unknown, reports range from 0.014% to 1.3% (reviewed by Oechslin and Jenni⁵⁸). Patients with LVNC tend to have early-onset disease, with clinical expression varying from asymptomatic to progressively poor cardiac function, ventricular hypertrophy, increased thromboembolic events, and SCD.⁶⁰ The left ventricle is typically affected, but 50% of patients with LVNC also have right ventricular involvement.^{61,62} Clinical manifestations and radiographic findings in LVNC can resemble those identified in DCM and can occur in conjunction with DCM or HCM in the same affected individual or family (reviewed by Oechslin and Jenni⁵⁸). There is ongoing controversy about whether LVNC is a distinct clinical entity. The World Health Organization lists LVNC as an unclassified cardiomyopathy,⁶³ as does a position statement from the European Society of Cardiology.⁶⁴ On the other hand, the American Heart Association classified LVNC as a primary genetic cardiomyopathy in 2006.⁶⁵

Genetic Etiology and Clinical Testing

Because LVNC is rare, its genetic etiology is not well understood. Variants have been described in known DCM and HCM genes encoding components of the sarcomere (*ACTC1*, *MYH7*, *MYBPC3*, and *TNNT2*),^{66–68} the Z-disk (*LDB3*),^{69,70} the nuclear lamina (*LMNA*),⁷¹ the dystrophin-associated glycoprotein complex (*DTNA*),⁷² as well as the Barth syndrome gene tafazzin (*TAZ*), a nuclear-encoded mitochondrial protein.⁷³ Of note, the *NKX2.5* gene (which encodes a cardiac specific transcription factor) had been implicated in the etiology of LVNC based on its presence in chromosomal region deleted in a girl with complex heart disease.⁷⁴ However, to date, no pathogenic variants in *NKX2.5* have been detected in individuals with isolated LVNC.

The role of genetic testing in patients with LVNC received the lowest grade based on the guideline published by the Heart Failure Society of America, recommending only variant-specific genetic testing for at-risk family members if a pathogenic LVNC variant is identified in the proband.⁴ Genetic testing is available simply because all genes associated with LVNC are also involved in other cardiomyopathies, for which testing has been more widely adopted. Detection rates have not yet been well defined and are currently restricted to isolated reports. In the Laboratory for Molecular Medicine, Partners HealthCare Center for Personalized Genetic Medicine, diagnostic testing of a broad referral population detected a clinically significant variant (classified as likely pathogenic or pathogenic) in 24% of 108 individuals with reported LVNC. Variants were present in *MYH7* (13.6%), *MYBPC3* (4.0%), *TNNI3* (2.0%), *VCL* (2.8%), *TAZ* (1.1%), and *TNNT2* (1.0%). (B. Funke, unpublished data). Splice variants in the *MYH7* gene, which are exceedingly rare in HCM and DCM, seem to be more prevalent in patients with LVNC⁶⁸ (B. Funke, unpublished observations).

Restrictive Cardiomyopathy

RCM is characterized by increased stiffness of the ventricular chambers, although ventricular wall thickness and systolic function is generally within normal limits. Most individuals with RCM develop heart failure and succumb to death within a few years.⁷⁵ Some reports suggest a clinical overlap between RCM and HCM.^{75–77}

Idiopathic RCM is rare, and its genetic etiology is only beginning to be defined. Recent studies report variants in sarcomere protein genes, including *TNNI3*, *TNNT2*, *MYH7*, and *ACTC1*.^{77–80} In addition, missense variants in the desmin gene (*DES*) have been identified in several families with desmin-related myopathy, which can present with RCM.⁸¹ Similar to LVNC, current Heart Rhythm Society/European Heart Rhythm Association guidelines recommend variant-specific genetic testing for at-risk family members after the identification of a pathogenic variant in the proband.⁴

In the Laboratory for Molecular Medicine, Partners HealthCare Center for Personalized Genetic Medicine, diagnostic testing of a broad referral population detected a clinically significant variant in 35% of 50 individuals with reported RCM. Variants were present in *TNNI3* (18%), *MYH7* (14%), and *MYBPC3* (2%) (B. Funke, unpublished data).

Genetic and Phenotypic Overlap between Inherited Cardiomyopathies

Cardiomyopathies have traditionally been defined based solely on clinical features, including ventricular morphology and function. Although HCM, DCM, and ARVC are generally recognized as distinct clinical entities, there is an increasing realization of substantial genetic and phenotypic

overlap. As described in the preceding sections, phenotypic overlap exists between end-stage HCM and DCM⁸² and between DCM and ARVC (which can manifest with ventricular dilatation and can have LV involvement).⁴⁷ Furthermore, features of LVNC can overlap with those of HCM, DCM, and RCM (reviewed by Oechslin and Jenni⁵⁸).

As the underlying genetic etiologies of these disorders are being elucidated, substantial and increasing overlap emerges (Table 1). For example, pathogenic sarcomere variants have been primarily identified in patients with HCM but, more recently, also in patients with DCM, LVNC, and RCM. Z-disk genes have been implicated in DCM and in HCM.⁸³ Desmosomal protein genes were initially thought to be involved only in ARVC, but recent evidence suggests that variants in these genes may also cause DCM.⁸⁴ The phenotypic spectrum of variants in the desmin gene includes DCM, RCM, and, most recently, ARVC.^{85–87} Finally, variants in the *TTN* gene were recently shown to be a frequent cause of DCM, but emerging evidence also associates this gene with ARVC.⁵⁵

Although it is clear that variants in a given gene can cause more than one cardiomyopathy, it has been debated whether the responsible variants are distinct. Some studies have identified the same variant in patients with HCM and in patients with DCM and have attributed this to phenotypic plasticity.^{35,88} However, the molecular mechanisms underlying HCM (increased contractility) and DCM (decreased contractility) are fundamentally different, which calls into question whether the same variant can truly cause both diseases. Functional characterization of several HCM and DCM variants indeed showed fundamentally opposite properties, supporting distinct variant spectra,^{89–92} as reviewed by Ashrafian and Watkins.⁹³ Assuming nonoverlapping variant spectra, the identification of HCM variants in patients with marked LV dilatation and impaired systolic function may, therefore, reflect end-stage remodeling of HCM rather than primary DCM. Another explanation for the identification of one and the same variant in diseases with different molecular mechanisms is that these are not the primary cause of disease but act as modifiers or are completely benign. We are only now beginning to appreciate the spectrum of rare benign variation, as it is beginning to be possible to query thousands of sequenced genomes and exomes (1000 Genomes Project, National Heart, Lung, and Blood Institute Exome Sequencing Project). These data sets have made it all too clear that in the past, many studies inferred pathogenicity based on insufficient evidence. This has recently been demonstrated for a large number of published variants that were reported as causative of DCM.⁹⁴ One example illustrating a variant's journey is the Ala833Thr variant in *MYBPC3*, which was initially reported in four probands with HCM and in 1 in 400 control individuals.^{18,95,96} The presence in a single control individual was regarded as insufficient to rule out a pathogenic role, as reduced penetrance is not uncommon in HCM. Today, we know that this variant is present in 12 of 6952 chromosomes (0.17%; National Heart, Lung, and Blood Institute Exome

Sequencing Project), illustrating the power of large genome sequencing studies and strongly suggesting that this variant is unlikely a primary cause of disease.

In summary, it seems likely that individual variants are most often specific to one cardiomyopathy presentation. However, further studies including careful phenotypic analysis and variant classification are necessary to establish this with certainty.

Genetic Testing for Inherited Cardiomyopathies in the Postgenomic Era

Until recently, comprehensive genetic testing for genetically heterogeneous diseases, such as inherited cardiomyopathies, was difficult. The high cost of traditional (Sanger) sequencing technology led to gene panels of limited size that typically did not accommodate genes with low detection rates. NGS technologies have now completely eliminated this limitation such that virtually any gene with a published association with the disease of interest can be tested. Whole exome and whole genome sequencing (WES/WGS) are the ultimate genetic tests, and early success stories provide a taste of their power.⁹⁷ However, the technical quality of WES/WGS is still suboptimal as coverage of essential genes cannot be guaranteed. In addition, despite the rapidly dropping cost of running the assay, the analysis of the enormous number of variants detected is still too complex to broadly offer WES/WGS, particularly when the clinical diagnosis is clear. For these reasons and at the time of writing this review, targeted NGS tests may be better first-line tests for patients with well-established clinical diagnoses. On the other hand, when this type of testing is negative or when a clinical diagnosis is ambiguous, WES/WGS are powerful tools and are being increasingly used in clinical practice.

Targeted NGS-based gene panels for inherited cardiomyopathies are offered by a growing number of laboratories in the United States and worldwide (<http://www.genetests.org> and <http://www.ncbi.nlm.nih.gov/gtr>). Although these panels are an order of magnitude smaller than WES/WGS-based testing (~50 to 100 genes), they are based on the same novel paradigm that the laboratory assay performed can include genes for more than one disease. The test ordered by the health care provider now becomes a virtual entity, triggering the analysis of specific sets of genes depending on the patient's diagnosis. This design provides optimal flexibility by enabling simultaneous screening of several sets of genes when the clinical diagnosis is uncertain but retains the option of analyzing disease-specific gene sets when the diagnosis is clear. This eliminates the costly and lengthy stepwise testing process that has dominated genetic testing for cardiomyopathies for a decade.

Despite these obvious advantages, NGS testing has some important limitations. In the past, the limited size of gene panels provided a natural selection for well-established disease genes with high detection rates. As this restriction

has been removed, it has become critical to carefully review the scientific evidence underlying a proclaimed disease gene. Genes with low detection rates are typically supported by a limited number of studies. In addition, many studies infer disease association based on a small number of variants and limited evidence, such as absence from a small number of healthy control individuals and evolutionary conservation of the affected amino acid. It has been estimated that screening 300 chromosomes identifies only 80% of all rare benign variation and that ≥ 6000 chromosomes are necessary to identify the full spectrum.⁹⁸ It is, therefore, not surprising that many cardiomyopathy variants initially published as causative turn out to be present at low frequencies in large exome sequencing data sets.⁹⁴ Additional genetic evidence, such as segregation with disease and functional data that would lend stronger support, is not easy to generate and is rarely available. *In vitro* functional data are frequently provided but have to be interpreted with caution as it may or may not translate into a clinical phenotype in humans. When critically evaluated, many cardiomyopathy genes are better classified as candidate genes. Table 1 lists established and proposed cardiomyopathy genes and classifies each gene into one of three levels of supporting evidence. Clinical sequencing of candidate genes that have not yet been firmly associated with disease can lead to an inflation of VUSs and, therefore, to an increased likelihood of receiving an inconclusive test result when no clearly pathogenic variant is present among the detected variants.

The Emerging Role of Molecular Diagnostic Testing Laboratories

Sequencing a large enough number of patient samples to definitively prove a disease association is difficult for any individual study, especially for genes with very low detection rates. In contrast, molecular diagnostic testing laboratories have the ability to accrue larger numbers of tested patients and can help shed light on the role of newly emerging disease genes. During the past decade, many laboratories have begun to populate this gray zone between clinical research and molecular diagnostic testing. Although testing laboratories typically do not have access to detailed clinical information, the sheer number of patients tested can help validate (or refute) published claims for a disease association. It is increasingly common that the genetic testing process involves a close collaboration between molecular geneticists and genetic counselors at the testing laboratory and the referring health care provider and the family. Genetic test results often initiate a cascade of events, including familial segregation studies and iterative reevaluation of clinical and molecular evidence, to determine the clinical significance of novel variants.

Although clinical sequencing of emerging disease genes is increasingly performed, it is important to realize that it can take years until the spectrum of pathogenic and benign variation has been characterized. Until then, testing often yields

many VUSs, and these inconclusive results can be problematic for the patient.⁹⁹ The rapidly increasing number of sequenced genomes is beginning to remedy this problem, as the spectrum of rare benign variation is better defined. However, several challenges remain. First, the lack of available clinical annotation for these cohorts can be problematic because reduced penetrance and late-onset disease is common for most cardiomyopathies. Therefore, very rare variants remain difficult to interpret as a presymptomatic status of the carrier cannot be excluded. Furthermore, the Single Nucleotide Polymorphism Database (dbSNP) is now well-known to contain clinically significant variants as more rare variation is deposited from broad populations and only some are adequately labeled as clinically significant. Finally, large-scale exome/genome sequencing efforts are still limited to a few ethnic groups, and the spectrum of common variants remains poorly defined for many minority populations.

Conclusions and Outlook

Testing for genetically heterogeneous cardiac disorders, such as inherited cardiomyopathies, has been challenging owing to several factors. First, the high cost of traditional sequencing technologies posed a severe limitation to the research and discovery of new disease genes and to the number of known disease genes that could be tested simultaneously in a clinical setting. In addition, although genetic testing has been recognized to be useful for predictive testing, our incomplete understanding of the spectrum of pathogenic variants and the high discovery rate of novel VUSs continue to limit the clinical utility of available tests. Finally, knowledge that cardiomyopathies have a significant genetic etiology has not yet been widely disseminated, especially for DCM, which was long believed to be mainly the result of environmental lesions.

Recent technological breakthroughs have now paved the way for the expanding adoption of genetic testing in clinical practice and the medical management of patients. NGS technologies have increased the size of genetic testing panels by an order of magnitude, and the \$1000 genome, which was unthinkable just 10 years ago, is now within reach. These developments are expected to radically change the landscape of medical genetic testing as we know it today. It is likely that with rapidly dropping test costs and increasing accuracy, WGS eventually will replace most, if not all, currently offered genetic tests. However, because the timely and accurate interpretation of the ~ 3 million variants present per genome is still challenging, WGS is currently used only for highly selected, clinically complex cases. Success stories where WGS identified the cause of such diagnostically challenging cases are inspiring and give an outlook to what will be common in the relatively near future. As we are beginning to sequence many genomes, we will undoubtedly identify genetic etiologies for inherited cardiomyopathies that have been elusive thus far.

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Supplemental Data

Supplemental material for this article can be found at <http://dx.doi.org/10.1016/j.jmoldx.2012.09.002>.

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