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Update 2011: Clinical and Genetic Issues in Familial Dilated Cardiomyopathy

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Abstract

A great deal of progress has recently been made in the discovery and understanding of the genetics of familial dilated cardiomyopathy (FDC). A consensus has emerged that with a new diagnosis of idiopathic dilated cardiomyopathy (IDC), the clinical screening of 1st degree family members will reveal FDC in at least 20-35% of cases. Point mutations in 31 autosomal and 2 X-linked genes representing diverse gene ontogeny have been implicated in causing FDC, but account for only 30-35% of genetic cause. Next generation sequencing (NGS) methods have dramatically decreased sequencing costs, making clinical genetic testing feasible for extensive panels of DCM genes. NGS also provides opportunities to discover additional genetic cause of FDC and IDC. Guidelines for evaluation and testing of FDC and IDC are now available, and when combined with FDC genetic testing and counseling will bring FDC/IDC genetics to the forefront of cardiovascular genetic medicine.

Keywords

cardiomyopathy; genetics; genetic counseling; genetic testing

Introduction

Since our 2005 review of familial dilated cardiomyopathy (FDC) genetics in this Journal (1), a great deal of additional progress has been made. We note other valuable dilated cardiomyopathy (DCM) reviews, consensus documents and guidelines since 2005 (2-17). We review key concepts of genetic research and provide recent updates in FDC genetics. We also note the dramatic innovation in sequencing technologies that are revolutionizing clinical and research genetic studies. Much of this is broadly applicable to all of cardiovascular genetics.

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Background: genetic studies, phenotype and genotype

Phenotype studies

In our prior review (1) we cited 19 DCM phenotype studies published between 1981 and 2003, principally focused on estimating the fraction of those patients with idiopathic dilated cardiomyopathy (IDC) who were found to have FDC using family history (FH) or clinical screening of family members. FDC is defined most conservatively as DCM meeting criteria for IDC in at least two closely related family members (1). Large retrospective studies in the 1980's estimated that 2-10% of individuals with IDC had FDC. In the 1990's studies involving larger cohorts of patients with IDC and prospective cardiovascular screening in their close relatives estimated that 20-48% of individuals with IDC could be shown to have FDC (18-20). A consensus has emerged that FDC will be found in at least 20-35% of those with IDC with clinical screening of first-degree family members, where clinical screening includes ECG and echocardiography or some other measure of LV size and function. Notably, a family history without clinical screening is much less sensitive to detect FDC (18).

Genetics studies

In 2005 we listed 19 genes shown to cause nonsyndromic DCM in humans (1). We now list 33 genes, 31 autosomal and 2 X-linked (Table 1) associated with DCM covering significant gene ontogeny (Table 2). Notably, the frequencies of DCM mutations in any one gene are low ($\ll 1\%$ to 6-8%), and a genetic cause is identified in only 30-35% of familial DCM cases (Table 1). In contrast, in HCM genetic cause can be found in 50-75% of familial cases, and in those cases when a mutation is identified, $>80\%$ can be found in one of two genes (*MYH7*, *MYBPC3*) (13). By inference from HCM (and LQTS, ARVD/C (13, 16)), FDC genetics are inherently more complex.

The number of DCM genes will continue to increase with ongoing discovery efforts. Also 'crossover' DCM phenotypes of desmosomal genes usually associated with ARVD/C present as DCM with low frequency (84); DCM phenotypes have also been observed for genes principally observed in HCM or the long QT syndrome, as previously reviewed (12, 13). We and others have recently shown that rare variant genetics are at play in some cases of peripartum cardiomyopathy (85-87).

What has not changed over the past 5 years

The core approach to human genetic studies remains the same: the careful and comprehensive phenotyping of subjects and their family members, and then correlating those phenotypes with genetic information. The challenge of this approach is to assure oneself that the genetic variation identified is causative of the phenotype of interest.

Gene mutations

The term 'mutation' is most commonly applied in Mendelian disease to one or a short string of variants in coding DNA (Table 3). The most common are missense mutations, but less common types include nonsense, splice site, and short insertion or deletion mutations (Table 3). Synonymous variants do not change the amino acid of that codon, while nonsynonymous variants do change the amino acid of that codon.

Classifying a variant as a disease-causing mutation

Ascertaining whether any one specific variant is causing the phenotype of interest requires weighing several types of evidence, and achieving a high level of certainty for any one variant is challenging, especially if that variant is novel (1, 88-90) (Table 3). In most cases,

the sum of all of the evidence is required to decide if the identified variants are relevant (Table 3). As noted below, with next generation sequencing (NGS) approaches, the unique genetic variants identified in an individual affected with a specific phenotype can be many – hundreds to thousands – creating new challenges.

Phenotypic considerations

The term ‘Mendelian disease’ has been applied to heritable genetic disease, usually familial, with identifiable inheritance patterns (dominant or recessive, and autosomal, X-linked or mitochondrial) (1). Many Mendelian diseases are uncommon to rare, with population frequencies well below 1%. For Mendelian disease demonstrating autosomal dominant inheritance (which is the case in most FDC families) (1), the most powerful evidence that a putative mutation is indeed disease-causing is segregation of the variant of interest with the disease phenotype in at least one large, multigenerational family with multiple affected individuals who carry the variant and multiple unaffected individuals who do not carry the variant (Table 3). Multiple large families available to assess segregation increases the strength of evidence. While this concept is superficially simple, certain features of adult-onset Mendelian disease commonly observed with FDC complicates this approach.

One feature is *incomplete penetrance*, which refers to individuals who carry a mutation but do not manifest any evidence of the disease phenotype. Thus, in gene discovery studies, the absence of a DCM phenotype in someone carrying a putative disease-causing variant can never be considered absolute evidence that the variant is not relevant: the individual in question may simply be manifesting incomplete penetrance. A key corollary for clinicians caring for at-risk family members is that a negative clinical cardiovascular evaluation at any age does not rule out the possibility that the family member may develop later disease. This provides the rationale for the periodic rescreening of at-risk family members who have normal evaluations.

A related concept, ‘age-dependent’ or ‘age-related’ penetrance, is also observed with FDC, where a disease-causing mutation usually manifests a disease phenotype only in the adult years, most commonly in the 4th to 6th decades or later.

Another feature that complicates FDC assessment is *variable expressivity*, which means that only some aspects of the DCM phenotype are present. For example, only mild left ventricular enlargement (LVE) without systolic dysfunction, or the onset of arrhythmia or conduction system disease with only borderline DCM may be observed. Also, age of onset can vary significantly, with variable severity of disease progression. Thus, within a large FDC family a wide range of clinical findings may be present without fully developed DCM. Reliance on endophenotypes (partial or sub-phenotypes) as an indication of genetic DCM/FDC also has been problematic, in part because subtle clinical changes may result from other more common causes of CV disease, making it difficult to decipher genetic from non-genetic cause.

While usually nonsyndromic, DCM can be included in syndromic disease involving various organ systems, but most commonly skeletal muscle disease (muscular dystrophy) (12).

Genotype considerations

Other criteria to assign causality (Table 3), in addition to segregation of the variant with the phenotype, include its relative rarity in control DNAs (commonly $\ll 1\%$). The rationale for this is that if it were common in the population, it would be unlikely to cause a rare genetic disease. Nevertheless, how rare is rare (<0.01 , <0.005 , <0.001 , <0.0001)? Some analyses have suggested that the majority of rare alleles (0.001 – 0.003) may be injurious (91). The caveat with control DNAs is that they should be representative of the race and/or ethnicity of

the DCM family, as variants observed to be common (>1%) in one population can be rare in a different population.

Conservation of the amino acid or nucleotide (i.e., lack of variation in the protein structure or specific nucleotide sequence (92, 93) of lower species) is also used to assess variants, with the rationale that an amino acid or a nucleotide position with greater variation in lower species may have increased tolerance to variants at that position and are therefore less likely to be disease-causing. Other features are also relevant (Table 3).

Much of this, vital for discovery efforts, is also relevant for FDC clinical genetics. These fundamental principles of human genetics investigations have not changed, but with NGS the quantity of data to which they are applied has changed dramatically.

Genetic counseling

Text limitations do not permit a reiteration of the components and importance of skilled genetic counseling, especially for difficult, confusing or syndromic cases, supported by geneticist consultations as needed (1). Unlike most cardiologists, genetic counselors are trained to deal with the family as a unit of inquiry rather than the individual patient, an essential quality for genetic medicine. Genetic counselors are also trained to emphasize disease prevention in contrast to the focus on disease treatment taken by most cardiovascular specialists. Both of these qualities are particularly relevant for facilitating genetic risk assessment. The availability of genetic counselors with cardiovascular training or experience can provide the support needed to initiate the practice of cardiovascular genetic medicine. We refer the reader to several citations that deal with these important points (1, 12, 15, 16, 94-96).

What has changed over the past 5 years

Sequencing methods

The most significant change is the dramatic improvement in efficiency and speed of gene sequencing methods. Next generation sequencing (NGS) is the term used to describe several diverse methods that improve sequencing throughput by several magnitudes, resulting in markedly reduced sequencing costs per nucleotide. This has led recently to sequencing the human exome routinely for research applications (97-99). The exome is defined as the protein coding portion (the exons) of the 18,000 – 19,000 genes, estimated at 1-2% of the human genome. NGS is also used to sequence the entire human genome (coding and non-coding regions of DNA), referred to as whole genome sequencing (WGS) (100). Because Mendelian disease typically affects the protein coding portions of the genome, exome sequencing is particularly relevant for rare variant Mendelian disease. As of 2010, typical costs of exome sequencing for research purposes are approximately \$2000 per DNA sample. New instruments and new methods for multiplexing DNAs on NGS instruments are being developed that will improve throughput and decrease cost, making <\$1000, or even <\$500 exome sequences likely in the near future. WGS charges on the open market now range from \$10,000 to \$20,000; these costs are also expected to decrease dramatically (10- to 20-fold) in the next few years, which will bring even WGS into the realm of clinical genetic testing, as well as within the domain of the NIH research budgets of many cardiovascular genetics studies. NGS, whether for exome or WGS, is dramatically transforming the experimental possibilities – study designs unthinkable even 1-2 years ago can now be proposed and attained (97-99).

Along with this rapidly expanding universe of opportunity from NGS will come monstrous quantities of human DNA sequence data, challenging the hardware and software of informatics platforms, and necessitating novel approaches to data assembly, storage and

analysis. Computational budgets for even modest exome projects now (terabytes of data) cost tens of thousands of dollars; larger projects containing hundreds to thousands of terabytes of data will require more robust outlays. These realities will require new 'pipelines' to be developed to efficiently analyze these massive data sets and reduce the cost of storage. This will also require new control DNA data sets to be generated, some of which is now underway (101).

Impact of NGS on clinical molecular genetic testing

NGS is directly related to the emergence of clinical genetic testing for FDC. As recently as 2-3 years ago, clinical genetic testing costing thousands of dollars was available only for a few HCM genes. Now panels of dozens of genes at reduced cost, incorporating many or all reported for any of the genetic cardiomyopathies (DCM, HCM, RCM, ARVD/C, and LVNC), are rapidly emerging using NGS methods. While this increase in data comes with a host of limitations and complications in interpretation, FDC testing sensitivity (the probability of finding a genetic cause with the genetic testing) now ranges from 15-25%, making pre-symptomatic testing feasible. Testing laboratories for DCM genes are catalogued at GeneTests (102), an online service hosted by NCBI.

The Genetic Information Nondiscrimination Act (GINA) of 2008

After many years of effort, a new federal law using the eponym of GINA now protects individuals from genetic discrimination in health care or employment. Further information is available at the National Genome Research Institute website (103).

Ongoing issues

Clinical progress

Despite the evidence supporting a genetic basis of IDC/FDC, the implementation of guidelines (13) by practitioners has been tepid. Adherence to such guidelines will require a shift in focus from strictly therapeutic measures for a single patient presenting with advanced disease to the consideration and assessment of DCM risk for an entire family (1, 13, 16) (Figure 1).

The rationale for these recommendations is that most IDC/DCM presents late in its causal pathway (advanced disease, usually with heart failure or sudden cardiac death), but early detection of asymptomatic DCM through screening enables presymptomatic intervention that may prevent or ameliorate the progression to advanced disease (95).

With a new IDC diagnosis, genetic risk evaluation should be initiated, including taking a 3-4 generation family history and recommending that 1st degree family members undergo clinical cardiovascular screening (Figure 1). Clinical genetic testing may also be warranted, including the competent interpretation of genetic results with appropriate counseling (1, 16). All of this may require a referral of patients to centers providing expertise in cardiovascular genetics and guidance on implementation of gene and/or mutation-specific therapies if indicated (13), ideally in centers with geneticists or genetic counselors working in collaboration with cardiologists in cardiovascular genetic medicine clinics (95).

How many genes might be involved in DCM?

Even though rare variants have been identified in >30 genes, we estimate that this accounts for only one-third of genetic cause of FDC. We predict this number will expand significantly. Discovery of additional genetic cause of DCM is still key to further understanding DCM genetics.

Genetic model for DCM

We have only scratched the surface in understanding DCM genetics. We have almost no insight into the causes of the marked variation in age of onset, disease penetrance, or clinical severity observed even for the same mutation within a large extended family, or between families with the same variant. Gene-environment interactions may explain some of this, but additional genetic variation may also explain a portion of this variability. Most of the FDC genetic data thus far supports a 'one gene' Mendelian model with marked locus (many genes) and marked allelic heterogeneity (many private mutations within any one gene). The impact of multiple mutations in the same individual has been recognized for HCM (104-109) and the long QT syndrome (108, 110), where two or more mutations have been shown to be associated with earlier onset and more severe disease in 3-7% of subjects. However, considerable additional genetic variation may be at play. Such genetic variation could include 'less common' common variants (e.g., allele frequencies 0.5 – 5%), additional rare variants (including the bi-allelic models as shown in HCM and ARVD/C (111, 112)), epigenetic factors, gene promoter site variants, or alterations in other genetically-driven regulatory processes such as microRNAs or their target sites. All of these remain to be evaluated for genetic DCM.

Is idiopathic dilated cardiomyopathy (IDC) a genetic disease?

A related issue involves the genetics of IDC, or DCM after all known causes (except genetic) have been ruled out, and its relationship to FDC. This is important because understanding the genetic basis of IDC could have a major public health impact, as non-ischemic DCM makes up a significant proportion of all forms of cardiomyopathy, and IDC is by far the largest component of nonischemic DCM. Here we differentiate a 'true' IDC as a patient with IDC who has had their first-degree family members clinically screened (history, exam, echocardiogram, ECG) to rule out FDC versus a 'presumptive' IDC – one who is negative for familial disease by a careful 3-4 generation family history but has not had family members screened beyond the FH. Preliminary data from our resequencing studies suggested that the frequency of possibly or likely disease-causing rare variants in a cohort of FDC and IDC probands (>300 in total) was similar (39, 113). However, in those studies the family members of the IDC probands were not systematically screened beyond FH, making it difficult to accurately assess the familial nature of disease in the 'apparently sporadic' IDC portion of our cohort. Therefore, whether 'true' sporadic IDC differs from FDC in gene composition, penetrance or expressivity remains untested in a large prospective study,

Summary

Recent progress for DCM genetics has been significant, although much remains to be learned. Clinical genetic testing is rapidly emerging, and NGS technology now permits patients to undergo clinical genetic testing for many genes at reduced cost. However, enthusiasm for DCM genetic testing remains tempered in 2011 in large part due to the testing sensitivity of 15-25%, and the plethora of DCM genes that makes the rare variants 'established as disease-causing' in any one gene only a very few. The discovery of new DCM genes and other DCM genetic cause, accelerated now by exome sequencing and soon by WGS, will lead to knowledge of the remainder of the genetic makeup of FDC and IDC. The careful and systematic phenotyping of DCM probands and family members, whether sporadic or familial, when combined with the cataloging of many DCM rare variants, will enable DCM genetics to move into the mainstream of cardiovascular genetic medicine.

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Abbreviations

ARVD/C	arrhythmogenic right ventricular dysplasia
DCM	dilated cardiomyopathy
IDC	idiopathic dilated cardiomyopathy
FDC	familial dilated cardiomyopathy
HCM	hypertrophic cardiomyopathy
LQTS	long QT syndrome
NGS	next generation sequencing
WGS	whole genome sequencing

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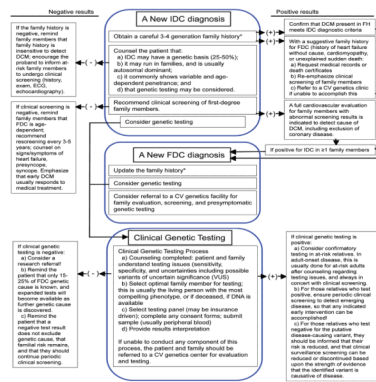


Figure 1. Flow diagram of genetic risk assessment for patients newly diagnosed with idiopathic dilated cardiomyopathy (IDC) or familial dilated cardiomyopathy (FDC)

The left- and right-sided boxes provide guidance for negative or positive results, respectively, based on the results of history or testing recommended in the central boxes. *Always search for history or exam findings consistent with syndromic disease, particularly skeletal muscle symptoms. However, with any suggestion of syndromic disease in the proband or family members, strongly consider referral to a geneticist or CV genetic medicine clinic with genetics collaboration. Some features of early onset conduction system disease or arrhythmia (usually from *LMNA* rare variants) may be particularly susceptible to genetic testing. Rare variants of unknown significance are not helpful for predictive testing.

Table 1

Genes reported in association with nonsyndromic dilated cardiomyopathy*

Gene [†]	Protein	Function [‡]	OMIM	Estimated fraction of DCM [†]	References
<i>LMNA</i>	lamin A/C	Structure/stability of inner nuclear membrane; gene expression	150330	0.06	(21-33)
<i>MYH6</i>	α -myosin heavy chain	Sarcomeric protein; muscle contraction	160710	0.043	(34, 35)
<i>MYH7</i>	β -myosin heavy chain	Sarcomeric protein; muscle contraction	160760	0.042	(36-39)
<i>MYPN</i>	myopalladin	Sarcomeric protein, z-disc	608517	0.035	(40)
<i>TNNI2</i>	cardiac troponin T	Sarcomeric protein; muscle contraction	191045	0.029	(36, 38, 39, 41-44)
<i>SCN5A</i>	sodium channel	Controls sodium ion flux	600163	0.026	(39, 45, 46)
<i>MYBPC3</i>	myosin-binding protein C	Sarcomeric protein; muscle contraction	600958	0.02	(35, 37)
<i>RBM20</i>	RNA binding protein 20	RNA binding protein of the spliceosome		0.019	
<i>TMPO</i>	thymopoletin	Also LAP2; a lamin-associated nuclear protein	188380	0.011	(47)
<i>LAMA4</i>	laminin a-4	Extracellular matrix protein	600133	0.011	(48)
<i>VCL</i>	metavinculin	Sarcomere structure; intercalated discs	193065	0.01	(38, 49)
<i>LDB3</i>	Cypher/ZASP	Cytoskeletal assembly; clustering of membrane proteins	605906	0.01	(39, 50)
<i>TCAP</i>	titin-cap or telethonin	Z-disc protein that associates with titin; sarcomere assembly	604488	0.01	(39, 51)
<i>PSENI/2</i>	presenilin 1 / 2	Transmembrane proteins, gamma secretase activity	104311/ 600759	0.01	(52)
<i>ACTN2</i>	α -actinin-2	Sarcomere structure; anchor for myofibrillar actin	102573	0.009	(53)
<i>CRYAB</i>	alpha B crystallin	Cytoskeletal protein	123590	0.007	(54)
<i>TPM1</i>	α -tropomyosin	Sarcomeric protein; muscle contraction	191010	0.006	(35, 55, 56)
<i>ABCC9</i>	SUR2A	Kir6.2 regulatory subunit, inwardly rectifying cardiac KATP channel	601439	0.006	(57)
<i>ACTC</i>	cardiac actin	Sarcomeric protein; muscle contraction	102540	0.005	(58-63)
<i>PDLIM3</i>	PDZ LIM domain protein 3	Cytoskeletal protein	605889	0.005	(64)
<i>ILK</i>	integrin-linked kinase	Intracellular serine-threonine kinase; interacts with integrins	602366	0.005	(48)
<i>TNNI1</i>	cardiac troponin C	Sarcomeric protein; muscle contraction	191040	0.004	(35, 43)
<i>TNNI3</i>	cardiac troponin I	sarcomeric protein, muscle contraction; also seen as recessive	191044	0.004	(35, 65, 66)
<i>PLN</i>	phospholamban	Sarcoplasmic reticulum Ca ⁺⁺ regulator; inhibits SERCA2 pump	172405	0.004	(38, 67-70)
<i>DES</i>	desmin	DAGC; transduces contractile forces	125660	0.003	(61, 71, 72)

Gene [†]	Protein	Function [‡]	OMIM	Estimated fraction of DCM ^{††}	References
<i>SGCD</i>	δ-sarcoglycan	DAGC; transduces contractile forces	601411	0.003	(72-74)
<i>CSRP3</i>	muscle LIM protein	Sarcomere stretch sensor/ Z discs	600824	0.003	(39, 75)
<i>TTN</i>	titin	Sarcomere structure/extensible scaffold for other proteins	188840	?	(76, 77)
<i>EYA4</i>	eyes-absent 4	Transcriptional co-activator	603550	?	(78)
<i>ANKRD1</i>	Ankyrin repeat domain-containing protein 1	cardiac ankyrin repeat protein (CARP); localized to myopalladin/titin complex	609599	?	(79)
<i>DMD</i> §	dystrophin	DAGC; transduces contractile force	300377	?	(80, 81)
<i>TAZ/G4.5</i> §	tafazzin	Unknown	300394	?	(82, 83)

* See Reference 12 for DCM associated with syndromic disease, and Reference 15 for genes not listed here that routinely cause combined skeletal and cardiac myopathies.

[†] Genes ordered by estimates of the fraction of DCM probands carrying mutations from primary and secondary reports; all are autosomal except as indicated; ? denotes inadequate data for estimate.

[‡] DAGC, dystrophin-associated glycoprotein complex.

§ X-linked genes.

Table 2

DCM Gene Ontology

Sarcomere	Cytoskeleton	Nuclear envelope
<i>ACTC</i>	<i>DMD</i>	
<i>MYH7</i>	<i>DES</i>	<i>LMNA</i>
<i>MYH6</i>	<i>LDB3</i>	<i>TMPO</i>
<i>MYBPC3</i>	<i>SGCD</i>	
<i>TNNT2</i>	<i>PDLIM3</i>	Gamma secretase activity
<i>TNNC1</i>	<i>VCL</i>	
<i>TNNI3</i>	<i>RYAB</i>	<i>PSEN1</i>
<i>TPM1</i>	<i>ILK</i>	<i>PSEN2</i>
<i>TTN</i>	<i>LAMA4</i>	
Z-disc	Mitochondrial	Sarcoplasmic reticulum
<i>TCAP</i>	<i>TAZ/G4.5</i>	<i>PLN</i>
<i>CSRP3</i>	RNA binding	Transcription factor
<i>ACTN2</i>	<i>RBM20</i>	
<i>MYPN</i>	Ion Channel	<i>EYA4</i>
<i>ANKRD1</i>	<i>ABCC</i>	
	<i>SCN5A</i>	

Table 3**Considerations for Molecular Genetic Testing**

A. Types of molecular genetic variants *	
Those affecting exonic (coding) sequence.	
Missense	Single base variant that changes an amino acid
Nonsense	Single base variant that changes an amino acid to a stop codon
Insertion/deletion (indel)	Usually one or a few nucleotides inserted or deleted. Unless the indel is in a multiple of three, a frameshift occurs that garbles the usual amino acid sequence. This usually results in an eventual stop codon.
Those affecting intronic or splice site sequences	
Splice site	Affects exon splicing; one or more exons may be skipped
Intronic	By definition intronic sequencing is non-coding. While intronic variation is more common than coding sequence, it has been infrequently associated with disease.

B. Testing categories of sequence variations relevant to a phenotype of interest (90)	
1	Sequence variation is previously reported and is a recognized cause of the disorder
2	Sequence variation is previously unreported and is of the type which is expected to cause the disorder
3	Sequence variation is previously unreported and is of the type which may or may not be causative of the disorder (also commonly referred to as a variant of unknown significance – VUS)
4	Sequence variation is previously unreported and is probably not causative of disease
5	Sequence variation is previously reported and is a recognized neutral variant
6	Sequence variation is not known or expected to be causative of disease, but is found to be associated with a clinical presentation

C. Criteria used to assess the relevance of a genetic variant for a phenotype of interest	
Property	Comment
Prior molecular genetic classification, if available	This may be definitive for variants previously established as disease-causing.
Type of variant (see Section A in this table).	A synonymous variant only in unusual circumstances is considered relevant for disease (e.g., a variant that opens a cryptic splice acceptor site).
Weight of evidence, in the gene in question, that rare nonsynonymous variants cause DCM.	**see comment below.
This is especially relevant for a novel gene under consideration in a discovery study. Disruption of a functional protein in the tissue of interest could lead to plausible pathophysiology.	Examples of established genes include those encoding proteins of the contractile apparatus (see Table 1). For discovery studies, evidence of cardiac expression or the presence of the protein product in cardiac tissue may aid in assessing relevance.
Rarity in the population	Many Mendelian variants may be 'private' or unique to a proband or family
Variant segregates with the DCM phenotype, ideally in one or more large families; lacking large families, the variant segregates with DCM in multiple smaller families, or is observed in multiple sporadic DCM cases.	In genetic DCM (and other multi-locus Mendelian diseases), many variants are 'private' so that multiple probands or families with any one specific variant are uncommon.
Functional data derived from the variant: cellular or animal models that recapitulate the	All model systems have inherent limitations and seldom provide definitive studies; however, such

disease phenotype

functional data increases the certainty that the variant under study is relevant for phenotype of interest.

* These variants do not account for copy number variants (CNV's; also termed structural variants), which are insertions, deletions, duplications or inversions of larger portions of DNA. CNVs range widely in size; from very small (fewer than a hundred nucleotides) to very large (many megabases), and all sizes in between. They may affect both coding and non-coding DNA. Structural variants are not detected by usual sequencing approaches. Systematic evaluation of structural variants has not been undertaken in DCM, and hence their relevance for DCM has not been established.

** Some genes (e.g., *LMNA*, *MYH7*, *TNNT2*, see Table 1) have abundant evidence that point mutations can cause DCM. Nevertheless, because of the marked allelic heterogeneity in DCM genes, it is uncommon for any one specific variant to be found in multiple unrelated probands, even in these genes. Whether any of these novel nonsynonymous rare variants can be considered disease-causing by usual molecular genetic diagnostic standards is an open question. Further, because most of the DCM genes (Table 1) have had only a few reported pathologic variants, newly identified rare variants in such genes with less prior DCM sequencing data available are commonly reported as variants of unknown significance (VUS; this table, Section B).