Genetic Evaluation of Dilated Cardiomyopathy

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Abstract Recent advances have expanded our ability to conduct a comprehensive genetic evaluation for dilated cardiomyopathy (DCM). By evaluating recent literature, this review aims to bring the reader up-to-date on the genetic evaluation of DCM. Updated guidelines have been published. Mutations in *BAG3*, including a large deletion, were identified in 2 % of DCM. Truncating mutations in *TTN* were reported in 25 % of DCM. Two new genes have been reported with autosomal recessive DCM. These studies illustrate the role of improved technologies while raising the possibility of a complex genetic model for DCM. The inclusion of *TTN* has led to an increased genetic testing detection rate of 40 %. While our ability to identify disease-causing variants has increased, so has the identification of variants of unknown significance. A genetic evaluation for DCM must therefore address this complexity.

Keywords Genetics · Dilated cardiomyopathy · Genetic counseling

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Introduction

Dilated cardiomyopathy (DCM) is one of the most common forms of cardiomyopathy with a prevalence likely exceeding 1/500 [1]. The diagnosis is based on increased left ventricular end diastolic diameter and systolic dysfunction. DCM can be present as an isolated finding or as part of a syndrome (Table 1). This review focuses on the genetic basis of nonsyndromic, isolated DCM. While most commonly evaluated by echocardiography, left ventricular enlargement (LVE) and systolic dysfunction may also be identified by left ventricular angiography or cardiac magnetic resonance imaging. LVE by echocardiography is deemed to be present when the diastolic left ventricular diameter exceeds the 95th percentile of a population-based height and gender-based approach [4]. Left ventricular dysfunction is defined as an ejection fraction of less than 50 % [5] (or more stringently, 45 % [6] or a fractional shortening of less than 25 %).

Other structural cardiac findings, such as right ventricular (RV) enlargement [7], fatty infiltration [8], and left ventricular noncompaction (LVNC) [9, 10] may also be present. DCM with RV involvement, however, must be differentiated from arrhythmogenic right ventricular cardiomyopathy (ARVC), a diagnosis involving right ventricular involvement, fibrofatty infiltration, prominent arrhythmia, and sudden death. LV involvement in ARVC, once thought to be part of the late phase of the condition [11], is also observed in ARVC with early, predominant LV involvement [12]. To account for this wide phenotypic spectrum, the more inclusive term "arrhythmogenic cardiomyopathy" has been proposed [13].

When symptomatic, DCM presents late in the disease course with heart failure (HF), arrhythmia, and conduction system disease (CSD; first, second, or third degree heart block) with or without sudden death, or with thromboembolism [14]. Because DCM is commonly asymptomatic until late in its disease course [15], it may only be identified

Table 1 Selected syndromic dilated cardiomyopathy causes

Syndrome	Locus	OMIM	Gene symbol	Gene product	Inheritance pattern*	Additional clinical features
Emery-Dreifuss muscular dystrophy (EDMD), limb girdle muscular dystrophy IB (LGMD 1B), Heart Hand Syndrome, Slovenian Type (HHSST)	1q21.2	150330	LMNA	Lamin A/C	EDMD: AD, AR; LGMD1B: AD; HHSST: AD	Joint contractures in EDMD; arrhythmias; childhood muscle weakness (shoulder/hip-girdle in LGMD1B; limb deformities in heart hand syndrome.
Hemochromatosis	6p21.3	35200	HFE	Hereditary hemochromatosis	AR	Cirrhosis, diabetes, hypermelanotic pigmentation, increased serum iron and ferritin.
Laing distal myopathy	14q12	160760	MYH7	ß-myosin heavy chain	AD	Childhood onset weakness of ankles and great toes, then finger extensors. Neck flexors and facial weakness.
Carvajal syndrome	6p24	125647	DSP	Desmoplakin	AR	Woolly hair, keratoderma.
Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD)	Xp21.2	300377	DMD	Dystrophin	XL	DMD: males: elevated CK, childhood muscle weakness, wheelchair bound by age 12, DCM after age 18; BMD: elevated CK, skeletal muscle weakness in 20's or lager; females can be affected with milder phenotype or DCM alone.
Barth Syndrome	Xq28	300394	TAZ/G4.5	Tafazzin	XL	Growth retardation, intermittent lactic acidemia, granulocytopenia, recurrent infections.
Kearns-Sayre syndrome	mtDNA multigene deletion	530000	Variable (eg, MTND5, MTND4, MTND3)	NADH dehydrogenase subunits 3, 4, & 5 others	De novo	Progressive external ophthalmoplegia, muscle weakness, cerebellar ataxia, diabetes mellitus.
Cantú syndrome	12p12.1	239850	ABCC9	subunit of ATP-sensitive potassium channel complex	AD	Congenital hypertrichosis, distinctive facial features, osteochondrodysplasia [2].
Congenital disorder of glycosylation	9q34.11	610746	DOLK	dolichol kinase, protein N-glycosylation	AR	Failure to thrive, mild developmental delay, hypotonia, short stature, ichthyosiform dermatitis, elevated transaminase [3].

AD autosomal dominant, AR autosomal recessive, XL X-linked

(Adapted from: Hershberger RE, Morales A, Siegfried JD. Clinical and genetic issues in dilated cardiomyopathy: a review for genetics professionals. Gen Med. 2010;12:655–67) [1]

serendipitously or as part of cardiovascular screening of at-risk family members. Onset usually occurs by age 60 [1], although it has been observed during the fetal stage [16], in infants, children [17•], and the elderly [8]. A subset of DCM, peripartum cardiomyopathy or pregnancy associated cardiomyopathy (PPCM/PACM), has been associated with pregnancy [18], although a causal role for pregnancy has yet to be demonstrated in humans. Features of LVNC have been reported in PPCM, and a familial basis has been suggested based on the transmission of LVNC from a mother with both PPCM and LVNC to her newborn child [19]. A true association between PPCM and LVNC needs to be confirmed with larger studies.

Management includes pharmacologic therapy with ACE inhibitors and beta blockers, and consideration of intracardiac defibrillator/pacemaker (ICD/PCM) for those with a history of or at risk for lethal arrhythmia. Advanced therapies, including left ventricular assist device and heart transplantation, are indicated for patients with severe or intractable disease. Pregnancy is contraindicated in women with DCM or who have been diagnosed with PPCM/PACM.

Etiology

A wide variety of etiologies underlie DCM. In the United States, ischemic disease from coronary artery disease (CAD) is the most common cause. Nonischemic disease follows, which encompasses a wide range of causes, including cardiotoxic drug exposure, endocrine disease, congenital heart defects, infections (viral, bacterial, parasitic, and fungal forms), and genetically-mediated forms. The term idiopathic dilated cardiomyopathy (IDC), coined before evidence of a genetic basis became available, refers to DCM in which all identifiable causes (excluding genetic) have been excluded. It is now known that a portion of IDC also includes genetic forms. For simplicity, and for the remainder of this review, the term DCM will be used for cases with known genetic cause (eg, *LMNA*-DCM, following a recent nomenclature recommendation)[1] and those in which ischemic, cardiotoxic, and infectious causes have been ruled out (also known as IDC).

Using cardiovascular screening, familial disease (familial dilated cardiomyopathy, FDC) is identified in up to 48 % of IDC [20–22]. In the clinical cardiovascular literature, pedigrees with more than 1 individual with IDC (multiplex pedigrees) are denoted as FDC while all other nonfamilial cases (simplex pedigrees) preserve their IDC categorization. Besides the observable difference in number of affected subjects when familial and simplex pedigrees are compared, the clinical presentation of DCM cases is the same, despite a familial or apparently sporadic nature [23].

Genetics

Mutations in over 30 genes of diverse ontology have been reported in DCM, with more convincing evidence supporting causation in familial cases [15]. Mutations in genes encoding proteins of the desmosome, traditionally associated with ARVC, are also present in DCM [24, 25, 26•], perhaps explaining the mixed ARVC/DCM phenotypes. Most mutations lead to an autosomal dominant pattern of inheritance, however, a minority are associated with recessive [27], X-linked [28], or maternal mitochondrial [29] forms, among which extra-cardiac features and early onset may be more frequent. Autosomal recessive phenotypes, which are more frequent in consanguineous families, have been reported with mutations in *TNNI3*, and more recently, with *GATAD1* (nonsyndromic DCM; Table 2) [30•] and *DOLK* (predominantly nonsyndromic DCM) [3•] mutations. Penetrance may be incomplete (the proportion of mutation-positive individuals who show the phenotype) and disease expression (the degree of severity among known affected, mutation-positive individuals) is variable. Various disease mechanisms are involved (depending on the mutation involved); however, myocardial injury is consistently the end-result [1]. At the population level, > 200 mutant alleles have been reported in the now >30 known genes [33], all leading to essentially the same clinical picture (allelic heterogeneity).

Missense, frameshift, nonsense, and small insertion/deletions mutations have been reported. The role of larger deletions in DCM was demonstrated in a gene discovery study that for the first time identified mutations in *BAG3* (BCL2-associated athanogene 3) as causative of DCM [31•] (Table 2). The study identified a whole exon deletion in *BAG3* in a large FDC pedigree and frameshift, nonsense and missense mutations in 2 % of DCM, including familial and simplex cases [31•]. *BAG3*, a 535 amino acid protein coded by 4 exons, is also implicated in severe dominant childhood muscular dystrophy [34]. The gene product works as a co-chaperone of heat shock proteins. Its binding to proteins related to DCM pathophysiology, such as Bcl-2 (a regulator of apoptosis), is thought to be disrupted in *BAG3*-DCM [31]. Larger deletions have been observed in *EYA4* [35] and *LMNA* [36], however, a multiplex ligation probe amplification screening of *LMNA* in 58 probands failed to identify copy number variation [37]. The prevalence of copy number variation in DCM thus remains unknown until more comprehensive studies are performed.

Truncating mutations in *TTN* (Table 2) have also been identified in approximately 20 % of cases [32•], thus, increasing the proportion of known genetic cause in DCM to 40 %. The clinical presentation of *TTN*-DCM does not appear to be different than that of other forms of genetic DCM. *TTN* is a key sarcomeric component that encodes the giant protein titin. It comprises 283 kilobases containing 363 exons coding for 38,138 amino acids [38]. *TTN* undergoes

Table 2 Newly reported nonsyndromic DCM genes

Gene	Locus	Genomic coordinates	Protein product	Function	OMIM	Comments	References
GATAD1	7q21.2	7:92,076,761–92,089,380	GATA zinc finger domain-containing protein 1.	regulates gene expression.	614518	Autosomal recessive inheritance in consanguineous family.	[30•]
BAG3	10q26.11	10:121,410,881–121,437,330	BCL2-associated athanogene 3.	co-chaperone of heat shock proteins.	603883	A whole exon deletion reported in large family..	[31•]
TTN	2q31	2:179,390,716–179,695,529	sarcomeric component.	striated muscle passive force, modulator of active contractile force.	188840	More evidence supporting pathogenic role of truncating mutations	[32•]

dramatic alternative splicing to produce many skeletal muscle and heart isoforms [39], and is regulated by *RBM20*, another gene associated with approximately 2 % of DCM [40–42]. Cardiac-relevant isoforms include N2B, N2BA, and novex-3 [32•]. *TTN* had been reported with DCM in early studies [43, 44], but the recent study is significant for using next generation sequencing technology to comprehensively evaluate *TTN* in a larger number of DCM probands ($n=203$) with both simplex and familial disease. The study, which suggested a causative role for *TTN* truncating mutations, led to the immediate addition of this gene to clinical genetic testing panels. However, because the recent study also identified *TTN* truncating mutations in 3 % of their 249 controls [32•], and because mutations are frequently novel, the complexity of adjudicating causality of genetic DCM variants has escalated to unprecedented levels. Furthermore, the Herman et al. study did not evaluate missense mutations, which are very common in most individuals [45].

Further complicating molecular interpretation is the finding of multiple mutations in DCM. Examples include FDC pedigrees with mutations in *LMNA* and *RBM20* [42], *MYH7* and *LDB3*, and *PSEN2,* and *SCN5A* [46]. Multiple mutations in simplex pedigrees have also been observed, including probands with mutations in *MYBPC3* and *TNNC1*, *TNNT2* and *TPM1*, *TNNC1* and *MYH7*, and a fourth one with *TCAP, LMNA,* and *MYH6* mutations [47]. Triple mutations were also seen in a proband with familial disease and 2 mutations in *MYH6* and 1 in *TNNT2* [47]. Compound heterozygosity was identified in a proband with familial disease and 2 different mutations in *MYBPC3* [47]. A homozygous *TNNT2* mutation was also observed in a simplex, consanguineous pedigree [46, 48]. It is difficult to sort out the relevance of each individual finding, as many of these mutations were novel while others were previously reported with DCM or other cardiovascular phenotypes. They were also rare in the general population and changed a key amino acid, all usual criteria to establish pathogenicity [15]. Therefore, the role of multiple mutations in DCM may be relevant [49], but has not been formally studied. A possible link between HCM and DCM, however, has been proposed based on multiple mutation findings [50].

Genetic Evaluation

In 2009, guidelines for the genetic evaluation of cardiomyopathy from the Heart Failure Society of America, including DCM, recommended family history, periodic cardiovascular screening of at-risk family members (by echocardiogram, electrocardiogram, and physical exam), and consideration of genetic testing with counseling for individuals with DCM, and, when applicable, their family members [51]. In families in which the disease-causing mutation is known, targeted

testing should be offered to at-risk family members. Those who test positive for the family mutation should undergo periodic screening on a yearly basis, while those who test negative for the family mutation may be discharged [51, 52]. The family members of probands in which genetic testing fails to identify a mutation or in which genetic testing has not been performed should undergo screening every 3–5 years [51].

More recent guidelines from the Heart Rhythm Society in conjunction with the European Heart Rhythm Association that included cardiomyopathy were published in 2011, which recommended *LMNA* and *SCN5A* genetic testing for individuals with DCM and significant CSD or premature, unexpected sudden cardiac death and targeted testing for at-risk family members when a mutation is identified in a proband [53•]. These guidelines also considered it helpful to offer genetic testing to individuals with FDC for diagnosis confirmation, identification of at-risk family members (including cases when previously undetected syndromic disease may be present), and for family planning. The guidelines also distinguished the clinical value of genetic testing for probands with and without CSD. For the former, genetic testing was deemed to have a greater impact, as genetic information can be useful not only for diagnostic purposes, but also for prognostic and perhaps therapeutic value, especially when mutations in *LMNA* and *DES* are present [53•]. For these individuals, prophylactic ICD/PCM may be considered.

Both guidelines, published before the *TTN* report [32•], do not fully address the genetic complexity of DCM and provide little insight to help navigate complex genetic testing reports in order to make results clinically useful. One of these issues is the likely finding of a variant of unknown significance (VUS). To illustrate this concept, before the recent *TTN* report, clinical laboratories were quoting a detection rate of approximately 20 %–40 %. However, an analysis of clinical genetic results concluded that disease causing mutations were present in 17.4 % whereas 10.6 % had a VUS [54•]. Many *TTN* variants, including truncating ones (also identified in 3 % of controls), fall into this category. That a VUS is a disappointing finding, and one that should be handled with care, is not a new concept; the novelty lies in the increased volume of VUSs that reports may now include, especially because all variants in the enormous gene *TTN*, including missense variants, are reported. Moreover, variants thought to be pathogenic may be reclassified as benign and vice-versa. These issues warrant careful discussion with patients during pre-test informed consent.

Genetic testing for DCM involves large gene panels. For DCM with more prominent than usual right ventricular (RV) involvement, or DCM with a prominent arrhythmogenic phenotype, multicardiomyopathy genetic testing panels including desmosomal genes can help to clarify diagnosis. All inclusive panels evaluating cardiomyopathy, channelopathy, and structural heart disease in a single test are also available, with some now including >50 genes. Careful review of the genes included in these panels is key to better address the evaluation needs of a patient. For example, some panels include mitochondrial genes (that may be more relevant in pediatric or multisystem involvement cases) while others do not. In addition, because gene panels are continually updated in response to gene discoveries or improved technology, a previously done panel may rapidly become outdated. Also, due to the possibility of multiple mutations, a panel may be offered to more than 1 affected family member (or even repeated, with an expanded number of genes, in a patient with a known gene mutation). Similarly, for families with mutations in genes with incomplete evidence for pathogenicity, such as *TTN*, alerting genotype- and phenotype-negative family members about signs and symptoms of DCM, and suggesting follow-up genetic evaluation later in life may be wise (until fully interpretable whole exome or whole genome testing becomes the standard approach to clinical genetic evaluations). The decision to repeat genetic testing panels or recommend additional clinical cardiovascular care should be exercised with caution, by expert cardiovascular genetics practitioners.

Until these issues are more appropriately addressed on the research arena with comprehensive sequencing of a much larger number of probands (and possibly with functional studies of key genes), clinical cardiovascular screening remains a reliable, yet more expensive, tool to evaluate all at-risk family members. The logical follow-up question would be how willing or able are patients to undergo clinical screening? This question was recently addressed in a retrospective chart review of 57 patients (11 DCM, 46 HCM) that reported an uptake of 57 % and a 25 % detection of familial disease [55•]. In this study, uptake of screening (proportion of at-risk first and second degree relatives who underwent echocardiogram and electrocardiogram) was more likely among first degree relatives of probands, relatives of probands with a known mutation, and in families with multiple affected living relatives. Among relatives of probands with a known mutation, the uptake of cardiac surveillance was higher (59 %) than the uptake of genetic testing (39 %). The authors speculated that the lower uptake of genetic testing may result from, among others factors, perception of high cost and fear of genetic discrimination [55•]. Although the results of this study should be viewed as preliminary due to the small sample size, especially for DCM, they provide a glimpse into the practical aspects of genetic evaluation guidelines for DCM.

To address these issues, providers may periodically recontact laboratories offering clinical genetic testing for DCM, as cost may no longer be an issue for some patients due to updated customer service policies. Patients should also be informed about the benefits and limitations of the Genetic Information Nondiscrimination Act (GINA) [56]. Discussing

that identifying silent disease through cardiac screening may have insurance implications may also help putting things in perspective. Patients may also benefit from visualizing the risk of genetic discrimination presented against the risk of not having a clear diagnosis or not being able to prospectively identify at-risk family members. For the reasons outlined above, informed consent for genetic testing should not only include technical information, but also a discussion to provide anticipatory guidance regarding potential reactions to results, discrimination risks, family communication issues, and cost, among others. For example, before testing begins, patients should be encouraged to picture different scenarios, including positive, negative, or uninformative results, and the possibility of results reclassification. In the setting of a positive result, duty to warn issues should be proactively addressed, so that ethical dilemmas related to results disclosure among family members may be avoided. Genetic counselors are uniquely equipped to assist patients and their families to explore these issues, which have been summarized in the setting of cardiovascular disease [57•].

A genetic evaluation should always begin with a careful and comprehensive phenotype, which includes a detailed, skillfully obtained family history by an informed professional [58, 59]. Current data supports panel genetic testing for the >30 genes in individuals with idiopathic dilated cardiomyopathy, preferably those with FDC. Cardiovascular data should be sought to confirm the phenotype. Similarly, especially given the complexity of genetic testing results, counseling of at-risk family members requires both phenotype (from recent cardiovascular screening) and genotype information. Those with suspected syndromic disease (eg, birth defects, abnormal stature, dysmorphic features, learning disabilities, multisystem involvement) should be evaluated by a clinical geneticist to rule out syndromic disease, for which other forms of genetic testing and management are more appropriate.

Conclusions and Future Directions

The field has reached a critical juncture in which sequencing technology may soon, if it has not already, outpace our ability to synthesize genetic data into clinically useful information. Although exome and whole genome sequencing, along with biomarker and environmental data, are essential to help solve these issues, managing large volumes of information using a multidisciplinary bioinformatics, clinical, and molecular approach will be key. Furthermore, deeper genome expeditions warrant at least equally deeper phenotype assessments to fulfill the promise of genomics and, ultimately, personalized medicine.

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