Published in final edited form as:

J Card Fail. 2012 April ; 18(4): 296–303. doi:10.1016/j.cardfail.2012.01.013.

Genetic Testing for Dilated Cardiomyopathy in Clinical Practice

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Abstract

Background—Familial involvement is common in dilated cardiomyopathy (DCM) and >40 genes have been implicated in causing disease. However, the role of genetic testing in clinical practice is not well defined. We examined the experience of clinical genetic testing in a diverse DCM population to characterize the prevalence and predictors of gene mutations.

Methods and Results—We studied 264 unrelated adult and pediatric DCM index patients referred to 1 reference lab for clinical genetic testing. Up to 10 genes were analyzed (*MYH7*, *TNNT2*, *TNNI3*, *TPM1*, *MYBPC3*, *ACTC*, *LMNA*, *PLN*, *TAZ*, and *LDB3*), and 70% of patients were tested for all genes. The mean age was 26.6 ± 21.3 years, and 52% had a family history of DCM. Rigorous criteria were used to classify DNA variants as clinically relevant (mutations), variants of unknown clinical significance (VUS), or presumed benign. Mutations were found in 17.4% of patients, commonly involving *MYH7*, *LMNA*, or *TNNT2* (78%). An additional 10.6% of patients had VUS. Genetic testing was rarely positive in older patients without a family history of DCM. Conversely in pediatric patients, family history did not increase the sensitivity of genetic testing.

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Disclosures

None.

Supplementary Data

Supplementary data related to this article can be found online at doi:10.1016/j.cardfail.2012.01.013.

Conclusions—Using rigorous criteria for classifying DNA variants, mutations were identified in 17% of a diverse group of DCM index patients referred for clinical genetic testing. The low sensitivity of genetic testing in DCM reflects limitations in both current methodology and knowledge of DCM-associated genes. However, if mutations are identified, genetic testing can help guide family management.

Keywords

Clinical genetics; heart failure; dilated cardiomyopathy; sarcomere genes; lamin A/C

Dilated cardiomyopathy (DCM) is an important cause of heart failure and the leading indication for cardiac transplantation worldwide.¹ With careful evaluation of relatives, familial disease can be identified in ~30% of patients with seemingly idiopathic DCM.² This connection indicates that genetic etiologies play an important role in disease pathogenesis. It also has important implications for families, because relatives are at risk for developing disease. Indeed, consensus guidelines for idiopathic DCM recommend that first-degree relatives of patients begin longitudinal echocardiography-based screening early in life.³ Therefore, greater understanding of the genetic basis of DCM will not only advance knowledge of fundamental disease biology, but will also improve clinical management of families by definitively identifying at-risk relatives, thereby allowing longitudinal screening to be focused only on mutation carriers.

Characterizing the genetics of DCM has been a challenging task owing to incomplete knowledge of the genes involved in disease as well as difficulties determining if DNA variants identified in patients are clinically significant. More than 40 different genes have been implicated in causing DCM. Unlike hypertrophic cardiomyopathy (HCM), which is largely a disease of the sarcomere, the pathways leading to DCM are considerably more diverse, involving genes encoding components of the sarcomere, Z-disk, nuclear lamina proteins, intermediate filaments, and the dystrophin-associated glycoprotein complex.⁴ Individually, each of these genes provides a very modest contribution to the overall incidence of disease, and efforts to identify other DCM-associated genes are ongoing. As a result, current genetic testing strategies are relatively insensitive in DCM. For example, the Familial Dilated Cardiomyopathy Project analyzed 14 genes in >300 index patients and identified disease-associated variants in only 20%–30% of cases.^{5–9}

Furthermore, even if genetic testing identifies a variant that differs from the expected/reference genetic sequence in a patient with DCM, it can be very difficult to determine whether the variant is a true pathogenic mutation capable of causing disease, is disease modifying, or merely represents benign genetic variation.^{10,11} This difficulty reflects the remarkable complexity in human genomic variation.¹² Correctly classifying DNA variants is obviously important to avoid false positive and negative genetic testing results, but there are no universal standards for interpretation. Interpretations are often based on only a small number of criteria, such as evolutionary conservation and absence of the DNA variant in a relatively small cohort of healthy control subjects.

In addition to a more complete compendium of genes that cause DCM, rigorous standardized metrics to classify DNA variants, and greater experience genotyping broader populations with disease are needed. Unlike earlier studies in which genetic testing was performed in carefully cultivated research cohorts, in the present study we assess genetic testing in real-life clinical practice. Using structured criteria to classify the significance of DNA variants, we describe the prevalence of gene mutations in a diverse population of DCM patients referred to a single laboratory for clinical genetic testing. We also examine factors that may influence the likelihood of identifying a mutation, to better characterize the

sensitivity, or yield, of genetic testing in the clinical setting. This information helps to shape the role of genetic testing in practice and to guide patient selection to maximize yield.

Methods

Subjects

The study cohort was drawn from all index patients referred for clinical DCM genetic testing from 2007 to 2009 to a single CLIA-certified reference lab, the Laboratory for Molecular Medicine (LMM) at the Partners Healthcare Center for Personalized Genetic Medicine, Cambridge, Massachusetts. As described below, because a number of different underlying diagnoses were indicated on the requisition forms, whenever possible, primary clinical information was obtained from referring physicians. A cardiologist with expertise in DCM and heart failure reviewed all data and independently adjudicated clinical diagnosis without knowledge of genetic testing results. Analyses were then restricted to patients with a standardized diagnosis of primary DCM.

Primary DCM was defined as systolic dysfunction (left ventricular [LV] ejection fraction <50%) with or without LV dilation¹³ in the absence of an apparent secondary cause of cardiomyopathy (eg, congenital heart disease, ischemic heart disease, uncontrolled hypertension, significant valvular disease). Subjects were excluded if they had an indeterminate or alternate clinical phenotype other than DCM (eg, myocarditis, hypertrophic cardiomyopathy, glycogen storage disease), a family history of HCM, or a subsequently identified sarcomere mutation known to cause HCM, or if there was LV non-compaction, arrhythmogenic right ventricular cardiomyopathy, Barth syndrome, or concomitant complex congenital heart disease.

This study was approved by the Partners Healthcare Institutional Review Board.

Clinical Data

Clinical information available on study subjects included date of birth, gender, race/ethnicity, vital status, clinical diagnosis, age at diagnosis (if unavailable, age at genetic testing was substituted), family history, cardiovascular history (myocardial infarction, hypertension, myocarditis, toxin/drug exposure), and cardiac structure and function, including maximal LV wall thickness, LV ejection fraction, and LV dimensions.

DNA Analysis

Genomic DNA was extracted from whole blood with the use of Puregene (Qiagen, Valencia, CA). All exons and splice regions were PCR amplified with the use of standard protocols (available on request) and sequenced on an ABI 3730 DNA analyzer (Applied Biosystems). Genetic testing was offered as a 2-tiered test (5 genes per tier), analyzing genes previously implicated in DCM. Tier 1 included 5 sarcomere genes: *MYH7* (beta myosin heavy chain), *TNNT2* (cardiac troponin T), *TPM1* (alpha tropomyosin), *MYBPC3* (myosin binding protein C), and *TNNI3* (cardiac troponin I). Tier 2 included *ACTC1* (alpha actin), *LMNA* (lamin A/C), *LDB3* (LIM domain-binding 3), *TAZ* (tafazzin), and *PLN* (phospholamban). Tests were typically performed in a reflexive manner such that tier 1 genes were initially analyzed. If no variants of interest were identified on tier 1, tier 2 genes would be analyzed.

Sequence analysis was performed with the use of Mutation Surveyor (Softgenetics, State College, Pennsylvania) and manually reviewed by 2 reviewers. All variants were confirmed by an independent Sanger sequencing assay.

Control Populations for Genetic Testing

Novel variants identified in Black DCM index patients were genotyped in 200 race-matched healthy control subjects. In the absence of race-matched control populations for Hispanic and Asian patients, novel variants identified in these populations were genotyped in Black as well as 200 White control subjects. Through ongoing experience with HCM genetic testing, the Laboratory for Molecular Medicine has sequenced sarcomere genes in over 1,000 White HCM index patients. A novel sarcomere variant in a White DCM index patient is therefore either unique to this patient or has a maximum frequency of 1/1,000 individuals. Therefore, it was not necessary to screen our (smaller) White control population for novel sarcomere variants identified in White DCM subjects in the present study, because their allele frequency can be assumed to be $\leq 1/2,000$. Controls were screened using Sequenom I-Plex technology (San Diego, California) followed by confirmatory Sanger sequencing of positive samples.

A detailed description of the control cohorts used can be found in the Supplemental Methods (available online at www.onlinejcf.com).

Classification of Variant Pathogenicity

Using a combination of methods, summarized in Supplemental Table 1 (available online at www.onlinejcf.com), the clinical significance of DNA variants was graded from 1 (benign) to 7 (pathogenic) with the following designations: grades 1–2: presumably benign; grades 3–4: variants of unknown significance; grades 5–7: clinically significant (see Supplemental Table 1 for details regarding criteria used for classification). Metrics for assessing the pathogenicity of variants included population frequency, evolutionary conservation, family evaluation/linkage/segregation, prior publications, functional studies, and computational (in silico) analysis. DNA sequence variants graded ≥ 5 are collectively referred to as “mutations” because they indicate DNA sequence variation that is likely to be clinically relevant. At the other end of the spectrum, variants graded 1 or 2 are presumed to be benign.

To be considered clinically relevant, at a minimum the variant had to be absent from control subjects and to affect evolutionarily conserved residues. This classification is based on the observation that the majority of missense variants have a deleterious effect and that a variant’s population frequency correlates with its potential clinical significance.^{14,15} De novo variants and novel variants that led to severe protein changes (eg, frameshift, nonsense, and splice variants) were also considered to be clinically relevant, provided that loss of function was a known disease mechanism for the gene. Mutations that segregated with disease in multiple individuals and/or were supported by strong functional data in addition to the criteria listed above were classified as pathogenic (grade 7).

DNA variants lacking sufficient evidence to be classified as clinically relevant (grades 3 and 4) were designated variants of unknown significance (VUS). These included novel variants identified in a minority-race patient without race-matched control subjects. This category also included variants that were absent from control subjects but exhibited other evidence that lowered the likelihood of being disease causing (eg, variants that altered less well conserved amino acids).

Variants in grades 1 and 2 included those present in $>1\%$ – 3% of the population. Additionally, synonymous DNA variants that do not affect amino acid coding, or those affecting noncoding (intronic) residues unrelated to gene splicing led to a presumed benign or benign classification. Common variants (population prevalence $\geq 1\%$ synonymous, $\geq 3\%$ nonsynonymous) are not further discussed in this manuscript.

Statistical Analysis

Normally distributed continuous data are presented as mean $\pm$ SD and compared with the use of Student *t* tests. Categorical variables are presented as frequencies and were analyzed with Fisher exact or chi-square tests. Multivariate logistic regression was used to test for demographic and clinical predictors of a positive genetic test result, and identify confounders and effect modification. All data were analyzed with SAS version 9.1 (SAS Institute, Cary, North Carolina).

Results

Clinical information was reviewed on 337 index patients referred for clinical DCM genetic testing by 95 providers. Seventy-three patients were excluded from analyses owing to an indeterminate phenotype or diagnosis other than DCM (eg, myocarditis, hypertrophic cardiomyopathy, glycogen storage disease, Barth syndrome). Thus, 264 DCM index patients were included in the present study, 91% of whom had LV dilation in addition to systolic dysfunction. The average age at diagnosis was 26.6 years (ranging from newborn to 71 years), and 42% were <18 years old. In accordance with laboratory practice at the time, genetic testing was performed as a 2-tier test (as described in the Methods) and included analysis of up to 10 genes. If a mutation was found on tier 1 (*MYH7*, *TNNT2*, *TPM1*, *MYBPC3*, and *TNNI3*), testing of tier 2 genes was not done. The majority of subjects (*n* = 187; 70%) underwent testing for all 10 genes; 14% had testing of only *MYH7*, *TNNT2*, *TPM1*, *MYBPC3*, and *TNNI3*, and the remaining 16% had selective genetic testing requested, typically targeting *LMNA*. The clinical characteristics of the 77 index patients who underwent partial genetic testing did not differ from the 187 who underwent complete (10 genes) testing (data not shown).

Results of Genetic Testing

Considering only variants considered to be clinically relevant (grades 5–7), “positive” genetic testing results were present in 17.4% of DCM index patients referred for clinical genotyping. The 40 clinically relevant variants identified in these 46 patients are listed in Table 1. Patients with negative genetic testing either had no variants identified, a VUS, or a (presumed) benign variant (Supplemental Tables 2 and 3, available online at www.onlinejcf.com).

Demographic and clinical characteristics of patients with positive and negative genetic testing are presented in Table 2. There was no significant difference in age at diagnosis, sex, or family history of DCM between index patients with positive and negative genetic testing. Mutations were most commonly identified in *MYH7* (6.6%), *LMNA* (5.3%), and *TNNT2* (3.7%). Collectively, mutations in these genes accounted for 78% of positive genetic tests (Table 3).

Combined clinical and genetic evaluations of family members were performed to assess the clinical significance of novel DNA variants. These segregation analyses supported pathogenicity in 8 novel variants and excluded pathogenicity of 3 *MYBPC3* variants (R1228C, E474D, S217G), because the variant was absent in affected family members (Fig. 1).

Variants of unknown significance were found in an additional 10.6% of index patients (*n* = 28), including 5 variants previously identified in HCM index patients. The clinical significance of these 5 variants remains uncertain for both cardiomyopathies, owing to a variety of reasons, including low evolutionary conservation of the affected amino acid (Supplemental Table 2).

Six patients (2.3%), aged 2 months to 17 years, carried >1 variant. Of those, 3 carried 1 mutation and 1 VUS. The remaining 3 carried 2 VUS (Supplemental Table 4, available online at www.onlinejcf.com).

Of the 11 likely benign variants, 7 have been previously published. One was reported as benign (*MYBPC3* G507R¹³), and 6 missense variants were reported as potentially disease causing in either HCM or DCM (*LDB3* D117N¹⁶; *MYBPC3* G278E,¹⁷ Q998E,^{18–20} V189I,²¹ A833T,^{7,18} and S217G²²). However, these 6 variants are more likely to be benign based on our criteria incorporating presence in healthy controls, frequent compound/double heterozygosity, lack of segregation with disease, and lack of evolutionary conservation.

Factors Associated With Positive Genetic Testing Results

Unadjusted comparisons revealed that age, sex, race, family history, and severity of LV systolic dysfunction were not significant univariate predictors of identifying a mutation (Table 2). However, age appears to influence the specific genes implicated in disease, as well as the relationship between family history and the likelihood of positive genetic test results. Adult-onset disease and family history of DCM were more common with *LMNA* than sarcomere mutations (mean age 45.0 vs 20.4 years [$P < .001$] and family history of DCM 70% vs 51% [$P = .15$] in *LMNA* versus sarcomere mutations). Sarcomere mutations were seen in patients of all ages, including 5 neonates.

Notably, family history had an age-dependent influence on the likelihood of identifying a DNA mutation. No mutations were identified in the 21 index patients >40 years old without a family history of DCM (Fig. 2).

Discussion

Clinical genetic testing results were positive in 17% of unrelated and unselected index DCM patients drawn from a diverse population in clinical practice. Mutations were most commonly seen in *MYH7*, *LMNA*, and *TNNT2*. This study extends the experience of earlier reports^{23,24} by evaluating genetic testing in a “real-life” setting. In contrast to other studies, the index patients included in this study were referred for clinical genetic testing as part of their management rather than being specifically recruited for research participation. They represented multiple ethnic backgrounds and spanned a wide age range, including pediatric DCM patients.

In contrast to HCM, in which a family history of disease substantially increases the likelihood of identifying a sarcomere mutation,⁵ family history alone was not strongly predictive of identifying a clinically relevant variant in DCM. However, age exerted an important influence. Genetic testing was frequently positive in young children with DCM in the absence of family history. This finding emphasizes the importance of considering genetic etiologies in all children who present with DCM. Conversely, no index patients >40 years old had a clinically relevant variant identified unless they also had a family history of DCM. Therefore, currently available genetic testing platforms may be less fruitful in patients who present as adults and do not have a family history of disease. A notable caveat to this observation is in patients who have clinical features to suspect lamin cardiomyopathy (prominent conduction disturbances and arrhythmias preceding development of DCM; skeletal myopathy) because *LMNA* variants typically present in adulthood.^{25,26} Moreover, undiscovered DCM disease genes may indeed play a role in adult-onset disease.

Although *MYBPC3* variants are an important cause of HCM, identified in >25% of HCM patients,¹⁷ our data suggest a more modest role for *MYBPC3* in DCM. Mutations in *MYBPC3* accounted for <1% of disease in this cohort. Earlier reports suggested that

MYBPC3 variants are relatively frequent in DCM;^{7,27} a conclusion that may reflect inappropriately including variants previously demonstrated to cause HCM as a cause of primary DCM. Given the considerable differences in natural history and histopathology between HCM and DCM, and their different consequences on sarcomere biophysical properties,²⁸ we suggest that it is unlikely that an individual sarcomere gene variant is capable of causing both HCM and primary DCM. Instead, the identification of HCM variants in patients with LV dilation and systolic dysfunction may reflect end-stage remodeling of HCM rather than primary DCM.

Challenges Facing Clinical Genetic Testing for DCM

Genetic testing for DCM is currently recommended in familial cases, where identifying the causal mutation can provide the opportunity for definitive risk assessment of healthy relatives through cascade testing.^{3,29,30} It can also be informative in patients with concomitant conduction disease, where identifying a genetic diagnosis can effectively rule out alternate diagnoses (ie, cardiac sarcoid). However, clinical sensitivity, ie, the likelihood that genetic testing will identify a clinically relevant DNA variant, is an important consideration to gauge the utility of genetic testing. In the present cohort, the overall sensitivity of genetic testing was relatively low, despite concentrating on patients without obvious confounding diagnoses and with a relatively high prevalence of familial disease—features that were expected to enrich for genetic disease.

The low yield of genetic testing is not unique to our cohort and is consistent with earlier reports.^{5,6} Several factors may contribute to a low detection rate. From a methodologic standpoint, we used Sanger sequencing, the current “gold standard” for clinical genetic testing. However, this technique cannot detect all types of genetic variation and may miss large insertions/deletions or copy number variations. Furthermore, from a biologic standpoint, DCM is substantially more heterogeneous than HCM, in which a relatively small number of genes account for a large portion of disease. Indeed, among HCM patients with positive genetic test results, ~80% have a mutation in either *MYBPC3* or *MYH7*.¹⁷ In contrast, >40 genes have been implicated in DCM, and clinically available genetic testing typically screens fewer than one-half of these.³¹ Incorporating ongoing gene discovery efforts and newer sequencing technologies³² into clinical laboratories will improve the sensitivity of genetic testing by allowing analyses of a larger number of DCM genes as well as detection of different types of genetic variation, without substantial increases in cost or time.

Determining if a DNA variant is the likely cause of disease is the most critical aspect of genetic testing. Clinically inconsequential DNA variants may be misclassified as pathogenic mutations without careful assessment of appropriate control data, familial segregation, and/or functional information. With this in mind, we used a rigorous approach to assess the clinical significance of DNA variants identified by genetic testing. Notably, this approach led to downgrading the clinical significance of several *MYBPC3* missense variants previously reported in the literature as pathogenic, but now revealed as likely benign. These variants were found in 4.5% of our index DCM patients. Had they been considered to be mutations, the sensitivity of genetic testing would have been falsely inflated by 21% (from 17.4% to 21.9%). At the individual level, without this critical reappraisal of variant pathogenicity, 12 additional patients would have been given false positive results, incorrectly implying that the DNA variant is responsible for disease. This outcome has particularly important implications for their families if cascade genetic testing is pursued. Healthy individuals could be mislabeled as being at risk by virtue of carrying the DNA variant, leading to unnecessary anxiety and clinical follow-up. Conversely, others that are truly at risk for developing disease could be falsely reassured. In reality, the genetic etiology of DCM in these families remains unknown, underscoring the importance for developing

standard criteria for defining the pathogenicity of DNA variants and interpreting genetic testing.

The absence of a variant from a control population and evolutionary conservation of the altered amino acid are commonly used to determine pathogenicity. However, emerging appreciation of the remarkable extent of human genetic variation suggests that 3,000 control individuals may be necessary to detect all benign rare variants in a population,³³ rather than the 100–200 control samples typically used for interpreting genetic test results. Although the present study is not immune to this limitation, the majority of our mutations have not been identified in >2,000 race-matched chromosomes.

To increase the clinical impact of genetic testing for DCM, many challenges must be met. The sensitivity of genetic testing will be improved by discovering additional DCM genes, developing more comprehensive test panels, and incorporating next-generation sequencing technologies to facilitate detection of the full repertoire of potential genetic variation with improved efficiency and cost.³² In addition to developing robust standards to classify the clinical relevance of DNA variants, new techniques are needed to allow functional testing of pathogenicity. Such techniques will allow rapid assessment of the functional impact of these variants on myocyte biology, improving the accuracy of interpreting genetic testing results. As technology and experience advances, it is important to bear in mind that interpretation of genetic testing is a dynamic process. As illustrated above, as new knowledge becomes available, the classification of previously identified variants may change. Fresh insights into variant pathogenicity may have important clinical implications; therefore, streamlined mechanisms are needed to notify practitioners when new data change the conclusions of earlier reports.³⁴ Meeting these challenges will enable patients and physicians to take advantage of the many potential benefits of gene-based diagnosis to identify individuals at risk for developing heart failure, to institute more intense clinical follow up and early treatment that may help diminish progression to symptomatic disease, and to better understand fundamental biology. Such opportunities will ultimately profoundly improve the care of patients and families with DCM.

Acknowledgments

The authors thank Scott Weiss for providing the Black control cohort and the Partners Healthcare Center for Personalized Genetic Medicine genotyping core laboratory for performing healthy control studies.

Funding: J. Ira and Nicki Harris Family Research Award (C.Y.H.), National Institutes of Health (C.E.S., J.G.S.), Howard Hughes Medical Institute (C.E.S.), and ACC/Merck Research Foundation (N.K.L.).

References

1. Taylor DO, Edwards LB, Boucek MM, Trulock EP, Aurora P, Christie J, et al. Registry of the International Society for Heart and Lung Transplantation: twenty-fourth official adult heart transplant reportd2007. *J Heart Lung Transplant*. 2007; 26:769–781. [PubMed: 17692781]
2. Mahon NG, Murphy RT, MacRae CA, Caforio AL, Elliott PM, McKenna WJ. Echocardiographic evaluation in asymptomatic relatives of patients with dilated cardiomyopathy reveals preclinical disease. *Ann Intern Med*. 2005; 143:108–115. [PubMed: 16027452]
3. Hershberger RE, Lindenfeld J, Mestroni L, Seidman CE, Taylor MR, Towbin JA. Genetic evaluation of cardiomyopathyda Heart Failure Society of America practice guideline. *J Card Fail*. 2009; 15:83–97. [PubMed: 19254666]
4. Dellefave L, McNally EM. The genetics of dilated cardiomyopathy. *Curr Opin Cardiol*. 2010; 25:198–204. [PubMed: 20186049]
5. Hershberger RE, Parks SB, Kushner JD, Li D, Ludwigsen S, Jakobs P, et al. Coding sequence mutations identified in *MYH7*, *TNNT2*, *SCN5A*, *CSRP3*, *LBD3*, and *TCAP* from 313 patients with familial or idiopathic dilated cardiomyopathy. *Clin Transl Sci*. 2008; 1:21–26. [PubMed: 19412328]

6. Parks SB, Kushner JD, Nauman D, Burgess D, Ludwigsen S, Peterson A, et al. Lamin A/C mutation analysis in a cohort of 324 unrelated patients with idiopathic or familial dilated cardiomyopathy. *Am Heart J*. 2008; 156:161–169. [PubMed: 18585512]
7. Hershberger RE, Norton N, Morales A, Li D, Siegfried JD, Gonzalez-Quintana J. Coding sequence rare variants identified in *MYBPC3*, *MYH6*, *TPM1*, *TNNC1*, and *TNNI3* From 312 patients with familial or idiopathic dilated cardiomyopathy. *Circ Cardiovasc Genet*. 2010; 3:155–161. [PubMed: 20215591]
8. Li D, Morales A, Gonzalez-Quintana J, Norton N, Siegfried JD, Hofmeyer M, et al. Identification of novel mutations in *RBM20* in patients with dilated cardiomyopathy. *Clin Transl Sci*. 2010; 3:90–97. [PubMed: 20590677]
9. Li D, Parks SB, Kushner JD, Nauman D, Burgess D, Ludwigsen S, et al. Mutations of presenilin genes in dilated cardiomyopathy and heart failure. *Am J Hum Genet*. 2006; 79:1030–1039. [PubMed: 17186461]
10. Hershberger RE, Siegfried JD. Update 2011: clinical and genetic issues in familial dilated cardiomyopathy. *J Am Coll Cardiol*. 2011; 57:1641–1649. [PubMed: 21492761]
11. Ho CY, MacRae CA. Defining the pathogenicity of DNA sequence variation. *Circ Cardiovasc Genet*. 2009; 2:95–97. [PubMed: 20031572]
12. Consortium TGP. A map of human genome variation from population-scale sequencing. *Nature*. 2010; 467:1061–1073. [PubMed: 20981092]
13. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association scientific statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation*. 2006; 113:1807–1816. [PubMed: 16567565]
14. Jordan DM, Kiezun A, Baxter SM, Agarwala V, Green RC, Murray MF, et al. Development and validation of a computational method for assessment of missense variants in hypertrophic cardiomyopathy. *Am J Hum Genet*. 2011; 88:183–192. [PubMed: 21310275]
15. Kryukov GV, Pennacchio LA, Sunyaev SR. Most rare missense alleles are deleterious in humans: implications for complex disease and association studies. *Am J Hum Genet*. 2007; 80:727–739. [PubMed: 17357078]
16. Vatta M, Mohapatra B, Jimenez S, Sanchez X, Faulkner G, Perles Z, et al. Mutations in Cypher/ZASP in patients with dilated cardiomyopathy and left ventricular noncompaction. *J Am Coll Cardiol*. 2003; 42:2014–2027. [PubMed: 14662268]
17. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, et al. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation*. 2003; 107:2227–2232. [PubMed: 12707239]
18. Van Driest SL, Vasile VC, Ommen SR, Will ML, Tajik AJ, Gersh BJ, et al. Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2004; 44:1903–1910. [PubMed: 15519027]
19. Garcia-Castro M, Coto E, Reguero JR, Berrazueta JR, Alvarez V, Alonso B, et al. Mutations in sarcomeric genes *MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, and *TPM1* in patients with hypertrophic cardiomyopathy. *Rev Esp Cardiol*. 2009; 62:48–56. [PubMed: 19150014]
20. Bahrudin U, Morisaki H, Morisaki T, Ninomiya H, Higaki K, Nanba E, et al. Ubiquitin-proteasome system impairment caused by a missense cardiac myosin-binding protein C mutation and associated with cardiac dysfunction in hypertrophic cardiomyopathy. *J Mol Biol*. 2008; 384:896–907. [PubMed: 18929575]
21. Girolami F, Passerini I, Nadalin N, Vargiu D, Cecchi F, Torricelli F. Myosin binding protein-C gene analysis by DHPLC: novel mutations and prevalence of compound heterozygosity associated with hypertrophic cardiomyopathy (Santorini Biologie Prospective Conference 2004). *Clin Chem Lab Med*. 2004; 42:A21–A60.
22. Mestroni L, Merlo M, Carniel E, Slavov D, Zhu X, Ferguson D, et al. Mutation screening of sarcomere genes *MYH7*, *MYBPC3*, *LDB3*, and *TNNT2* in a large cohort of dilated cardiomyopathy families. *Am Coll Cardiol*. 2010; 55 A31.E293.

23. Kushner JD, Nauman D, Burgess D, Ludwigsen S, Parks SB, Pantely G, et al. Clinical characteristics of 304 kindreds evaluated for familial dilated cardiomyopathy. *J Card Fail.* 2006; 12:422–429. [PubMed: 16911908]
24. Shimizu M, Ino H, Yasuda T, Fujino N, Uchiyama K, Mabuchi T, et al. Gene mutations in adult Japanese patients with dilated cardiomyopathy. *Circ J.* 2005; 69:150–153. [PubMed: 15671604]
25. Arbustini E, Pilotto A, Repetto A, Grasso M, Negri A, Diegoli M, et al. Autosomal dominant dilated cardiomyopathy with atrioventricular block: a lamin A/C defect-related disease. *J Am Coll Cardiol.* 2002; 39:981–990. [PubMed: 11897440]
26. Fatkin D, MacRae C, Sasaki T, Wolff MR, Porcu M, Frenneaux M, et al. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. *N Engl J Med.* 1999; 341:1715–1724. [PubMed: 10580070]
27. Zimmerman RS, Cox S, Lakdawala NK, Cirino A, Mancini-DiNardo D, Clark E, et al. A novel custom resequencing array for dilated cardiomyopathy. *Genet Med.* 2010; 12:268–278. [PubMed: 20474083]
28. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and α -tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. *Circ Res.* 2007; 101:1266–1273. [PubMed: 17932326]
29. Lakdawala NK, Givertz MM. Dilated cardiomyopathy with conduction disease and arrhythmia. *Circulation.* 2010; 122:527–534. [PubMed: 20679582]
30. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Heart Rhythm.* 2011; 8:1308–1339. [PubMed: 21787999]
31. Hershberger RE, Lindenfeld J, Mestroni L, Seidman CE, Taylor MRG, Towbin JA. Genetic evaluation of cardiomyopathy: a Heart Failure Society of America practice guideline. *J Card Fail.* 2009; 15:83–97. [PubMed: 19254666]
32. Herman DS, Hovingh GK, Iartchouk O, Rehm HL, Kucherlapati R, Seidman JG, et al. Filter-based hybridization capture of subgenomes enables resequencing and copy-number detection. *Nat Methods.* 2009; 6:507–510. [PubMed: 19543287]
33. Ionita-Laza I, Lange C, Laird NM. Estimating the number of unseen variants in the human genome. *Proc Natl Acad Sci U S A.* 2009; 106:5008–5013. [PubMed: 19276111]
34. Kohane IS, Mandl KD, Taylor PL, Holm IA, Nigrin DJ, Kunkel LM. Medicine: reestablishing the researcher-patient compact. *Science.* 2007; 316:836–837. [PubMed: 17495156]

Fig. 1.

Family genetic studies indicate that 3 *MYBPC3* variants previously reported to be pathogenic do not cause dilated cardiomyopathy (DCM). Combined clinical and genetic evaluation (segregation studies) provided valuable information in assessing the likelihood that a DNA variant causes disease. The absence of the variant in affected family members (*circled*) provides strong evidence that the variant is not pathogenic. Information supportive of phenotype is provided below the circled nonsegregant individuals. Filled symbols indicate affected individuals, squares males, circles females, arrows the index patient for genetic testing. Genotypes (+ or -) indicate the presence or absence of the familial variant. Dx, age at diagnosis; HCM, hypertrophic cardiomyopathy; LV mod dil, moderate left ventricular dilation.

Fig. 2.

Family history (FH) and age influence the sensitivity of genetic testing. In the absence of a family history of disease, no adult dilated cardiomyopathy patients >40 years old were found to have a mutation. Seventeen patients with unknown family history were excluded.

*Mutation refers to the presence of a clinically significant variant.

Table 1

Clinically Significant DCM Mutations

Gene	Exon/Intron	DNA	Protein	Patients, N	Segregation *	Grade †
MYH7	12	1106G>A	R369Q	2	DN	6
MYH7	13	1157A>G	Y386C	1		5
MYH7	14	1402T>C	F468L	1		5
MYH7	14	1405G>T	D469Y	1	1	5
MYH7	15	1573G>A	E525K	1	DN	6
MYH7	21	2348G>C	R783P	1	DN	6
MYH7	22	2678C>T	A893V	1	3	6
MYH7	23	2711G>A	R904H	1	DN	6
MYH7	25	3152C>T	A1051V	1		5
MYH7	27	3578G>A	R1193H	1	1	6
MYH7	29	3856G>A	E1286K	1		5
MYH7	30	4030C>T	R1344W	1		5
MYH7	31	4348G>A	D1450N	1		5
MYH7	37	5380C>G	Q1794E	1		5
MYH7	39	5740G>A	E1914K	1		5
LMNA	01	0348_349insG	K117fs	1		6
LMNA	01	215G>T	R72L	1	2	6
LMNA	01	0154C>G	L52V	1	4 [‡]	6
LMNA	03	607G>A	E203K [‡]	1		7
LMNA	04	799T>C	Y267H [‡]	1	5 ^{‡,p}	7
LMNA	06	1111_1125del	M371_A375del	1	DN	6
LMNA	06	958delC	L320fs	1		6
LMNA	06	1003C>T	R335W	2		6
LMNA	08	112G>A	R471H	1		6
LMNA	10	1621C>T	R541C	1		6
TNNT2	08	249G>C	E83D	1		5
TNNT2	08	218A>G	N73S	1		5
TNNT2	10	400C>G	R134G	1	2 [‡]	6

Gene	Exon/Intron	DNA	Protein	Patients, N	Segregation*	Grade [†]
TNNT2	10	421C>T	R141W [‡]	1		7
TNNT2	11	518G>A	R173Q	1		6
TNNT2	13	629_631del	K210del	4	1	7
TPM1	01	23T>G	M8R	1	DN	6
TPM1	07	688G>A	D230N [‡]	2	13	7
TPM1	06b	632C>G	A211G	1		5
ACTC1	02	383C>T	T128I	1	3	6
ACTC1	04	756T>G	I252M	1		5
MYBPC3	02	239delCinsGAGG	A80delinsGG	1		6
MYBPC3	28	2909G>A	R970Q	1		5
TNNI3	07	544G>A	E182K	1	DN	6
TNNI3	08	550G>A	E184K	1	DN	6

DN, de novo variant not present in either parent of the index patient.

[‡]Published data, citations for previously published variants found in the supplemental materials (available online at www.onlinejcf.com).

* Number of affected genotype positive family members.

[†]Variant classification grade as described in methods. Grades 5–7 were considered to be clinically relevant mutations; grade 7 indicates highest evidence to support variant pathogenicity.

[‡]Includes obligate carrier/s.

Table 2

Demographic and Clinical Features of the Study Cohort

	Mutation [*] Identified (n = 46)	No Mutation [†] Identified (n = 218)	P Value
Age at diagnosis, y, mean (range)	25.8 ± 18.6 (0–64)	26.8 ± 21.8 (0–71)	.77
Female, %	43	37	.44
Race, n (%)			.41
White	31 (67)	128 (59)	
Black	2 (4)	19 (9)	
Hispanic	4 (9)	13 (6)	
Asian	2 (4)	3 (1)	
Other/unknown	7 (15)	53 (25)	
Family history of DCM, %	55	51	.66
LVEF, %	28.1 ± 14.3	29.6 ± 13.6	.67

DCM, dilated cardiomyopathy; LVEF, left ventricular ejection fraction (available in 104 patients).

^{*} Mutation refers to the presence of a clinically significant variant (variant grade 5–7 by our classification criteria).

[†] Includes negative test and variants of unknown clinical significance (grade 4 by our classification criteria).

Table 3

Distribution of Clinically Significant DNA Variants Identified by Gene

Gene	No. of Patients Tested	Mutations, * % (n)
<i>MYH7</i>	241	6.6 (16)
<i>TNNT2</i>	241	3.7 (9)
<i>TPM1</i>	240	1.7 (4)
<i>MYBPC3</i>	240	0.8 (2)
<i>TNNI3</i>	240	0.8 (2)
<i>ACTC</i>	222	0.9 (2)
<i>LMNA</i>	208	5.3 (11)
<i>LDB3</i>	201	0 (0)
<i>TAZ</i>	203	0 (0)
<i>PLN</i>	200	0 (0)
Overall	264	17.4 (46)

Six variants identified in multivariant cases were not included, to avoid double counting.

* Mutation refers to the presence of a clinically significant variant (grade 5 by our classification criteria).