

ORIGINAL RESEARCH

Retrospective Analysis of Clinical Genetic Testing in Pediatric Primary Dilated Cardiomyopathy: Testing Outcomes and the Effects of Variant Reclassification

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BACKGROUND: Genetic testing in pediatric primary dilated cardiomyopathy (DCM) patients has identified numerous disease-causing variants, but few studies have evaluated genetic testing outcomes in this population in the context of patient and familial clinical data or assessed the clinical implications of temporal changes in genetic testing results.

METHODS AND RESULTS: We performed a retrospective analysis of all patients with primary DCM who presented to our institution between 2008 and 2018. Variants identified by genetic testing were reevaluated for pathogenicity on the basis of current guidelines for variant classification. A total of 73 patients with primary DCM presented to our institution and 63 (86%) were probands that underwent cardiomyopathy-specific gene testing. A disease-causing variant was identified in 19 of 63 (30%) of cases, with at least 9/19 (47%) variants occurring de novo. Positive family history was not associated with identification of a causal variant. Reclassification of variants resulted in the downgrading of a large proportion of variants of uncertain significance and did not identify any new disease-causing variants.

CONCLUSIONS: Clinical genetic testing identifies a causal variant in one third of pediatric patients with primary DCM. Variant reevaluation significantly decreased the number of variants of uncertain significance, but a large burden of variants of uncertain significance remain. These results highlight the need for periodic reanalysis of genetic testing results, additional investigation of genotype-phenotype correlations in DCM through large, multicenter genetic studies, and development of improved tools for functional characterization of variants of uncertain significance.

Key Words: familial dilated cardiomyopathy ■ genetic testing ■ idiopathic dilated cardiomyopathy ■ variant of uncertain significance ■ variant reanalysis

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Dilated cardiomyopathy (DCM) is the prevalent form of pediatric cardiomyopathy and results from intrinsic or extrinsic insults to cardiomyocyte mechanics, calcium signaling, and downstream intracellular signaling pathways.^{1,2} In large prospective studies of pediatric cardiomyopathy, 30% to 50% of DCM cases have an identifiable etiology, with neuromuscular disease and myocarditis being the most common causes, and the remaining 50%–70% of cases are categorized

as idiopathic.^{3–7} More recently, improvement in the ability to determine an etiology in patients with DCM following a formal genetic and metabolic clinical evaluation have been reported⁸; however, nearly half of all cases remain without a clear cause, even upon evaluation for copy number variants and causal single-nucleotide variants by whole exome sequencing.⁹

Pathogenic variants that result in a DCM phenotype have been identified in proteins that affect cardiomyocyte

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CLINICAL PERSPECTIVE

What Is New?

- Reclassification of previously identified variants results in downgrading of ≈30% of variants of uncertain significance.
- Many cases of pediatric primary dilated cardiomyopathy with an identifiable genetic cause arise from de novo mutations.

What Are the Clinical Implications?

- The majority of cases of pediatric primary dilated cardiomyopathy have no identifiable genetic etiology on gene panel testing.
- Many patients who have previously undergone genetic testing may benefit from reanalysis of testing results or updated testing.
- Cascade screening of family members improves variant interpretation and identification of disease-causing variants.

Nonstandard Abbreviations and Acronyms

DCM	dilated cardiomyopathy
LB	likely benign
LP	likely pathogenic
VUS	variant of uncertain significance

ultrastructure, sarcomeric integrity, force generation, cellular metabolism, and transcriptional regulation.^{10–12} A majority of the causal variants reported in the literature have been identified in adult patients, and only a limited number of studies have evaluated the genetic basis of DCM in the pediatric population.^{13–15} An estimated 25% to 50% of DCM cases in the adult population are explained by disease-causing mutations,¹⁶ and a similar rate has been reported in small pediatric cohorts.^{9,17,18} Genetic studies that have identified variants in pediatric DCM patients have demonstrated significant overlap in the affected genes with adult DCM studies but have also identified key age-related differences in the onset of phenotype based on the affected gene.^{11,17} Genetic testing is now recommended as part of the evaluation of all patients with cardiomyopathy,¹⁹ but the prognostic utility of this information, especially within the pediatric population, remains limited.^{20,21}

In this study, we review our institutional experience with clinical genetic testing of pediatric probands with primary DCM. By describing the phenotypic characteristics of these patients and evaluating temporal changes in testing results, we aim to better understand the diagnostic utility of genetic testing in this population

and the contribution of testing results to overall patient care.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patient Identification and Diagnostics

Patients were retrospectively identified by querying records between January 1, 2008, and January 1, 2018, for the diagnostic Fyler code for DCM. In general, this code is assigned at our institution on the basis of the presence of structural characteristics of DCM, a dilated left ventricle with systolic dysfunction. Patients with secondary DCM or incomplete clinical documentation were excluded. Three patients with borderline inflammation on endomyocardial biopsy who were subsequently diagnosed with DCM were included (Figure S1). Positive family history was defined as the presence of cardiomyopathy in a first-degree relative or multiple (≥2) second- and third-degree relatives with cardiomyopathy or sudden cardiac death. All functional cardiac data were calculated from the patient's echocardiogram at the time of initial presentation. Ejection fraction was calculated using the 5/6 area-length method.

Genetic Testing

Clinical cardiomyopathy genetic testing was performed at the Partners Healthcare Laboratory for Molecular Medicine (Cambridge, MA) as previously described.¹¹ The number of genes sequenced per patient and sequencing methodology differed over the course of the study period. There was an expansion in the number of genes included in the testing panel from 5 to 62, and 23 patients underwent testing with a next-generation sequencing–based panel composed of 62 genes associated with cardiomyopathy (Table S1). Variants were classified at the time of initial testing as previously described.^{11,22} Given significant changes in cardiomyopathy testing panel content, revisions in guidelines for variant classification, and an increase in the availability of reference population allele databases over the 10-year study period,²³ variants were reclassified in accordance with the American College of Medical Genetics and Genomics and the Association for Molecular Pathology Guidelines²⁴ and additional specifications for cardiovascular genes as established by the Clinical Genome inherited cardiomyopathy expert panel.²⁵ Gene-specific testing was performed in a subset of parents and siblings of probands for segregation analysis.

Statistical Analysis

Continuous variables are presented as medians with ranges, and a Wilcoxon rank sum test was used for comparison. Categorical variables are displayed as frequencies and percentages, and Fisher's exact test was used for statistical comparison. Survival analyses were performed with the Kaplan-Meier method and were divided according to age (<1 year and >1 year) and the presence or absence of a DCM-causing genetic variant. For transplant-free survival analysis, end points of heart transplantation or death were used and subjects were censored at the time of last follow-up. For transplant-censored survival analysis, a single end point of death was used, and patients were censored at the time of heart transplantation or time of last follow-up. Pairwise comparison of survival curves was performed with a log-rank test. Multivariate Cox proportional hazard modeling was performed to determine the association between survival time and covariates of sex, age, and presence of a DCM-causing variant. A $P < 0.05$ was considered statistically significant. Statistical analysis was performed in R 3.5.1.

Institutional Approval

All data collection, storage, and analysis was approved by the Institutional Review Board at Boston Children's Hospital and Partners Healthcare. Requirement for informed consent was waived by the Institutional Review Board.

RESULTS

Patient Demographics

We identified 118 patients with DCM who were evaluated at our tertiary care center over a 10-year period (Figure S1). The etiologies of DCM in this cohort were similar to those described in larger case series,⁴ with 80 patients having familial (12%) or idiopathic (56%) DCM and the remainder of cases caused by neuromuscular disease (6%), acute myocarditis (8%), metabolic disorder (10%), and genetic syndrome (8%) (Figure S1). Of the 80 patients who presented with primary DCM, 73 were probands with no prior genetic evaluation, and 63 (86%) of these patients underwent cardiomyopathy-specific gene testing as part of their diagnostic evaluation. The median age at the time of presentation for probands that underwent genetic testing was 6.5 years (range, 0–22) and 37 of 63 (59%) were male (Table 1). There was a bimodal distribution of age at the time of presentation, with 35% of patients presenting before the age of 1 year, and a second

group of patients presenting between the ages of 13 and 19 years. Eleven of 60 (18%) probands with available family history data had a positive family history of cardiomyopathy or sudden death, and all patients with a positive family history presented after 1 year of age ($P = 0.005$). The median left ventricular ejection fraction at the time of presentation was 26% (range, 8–65) and tended to be lower in those patients presenting under 1 year of age (23% versus 29.5%; $P = 0.06$). The median left ventricular end-diastolic volume z score at the time of presentation was +5.85 (range, –0.1 to +21.1) and did not differ between age groups (Table 1). One individual presented with a decreased ejection fraction without ventricular dilation, and another individual with ventricular dilation with a normal ejection fraction, both of whom went on to have DCM.

Genetic Evaluation of Patients With DCM

Cardiomyopathy gene panel testing of 63 probands resulted in the identification of 116 variants that were classified at the initial time of testing as follows: 8 pathogenic, 11 likely pathogenic (LP), 90 variants of unknown significance (VUSs), 3 likely benign (LB), 2 benign, and 2 unclassified on the basis of their position in untranslated regions (Table 2). The burden of VUSs per patient correlated with the size of the testing panel as previously described (Table S2).^{11,18} Following variant reevaluation for this study, the 116 variants were classified as 7 pathogenic, 8 LP, 68 VUSs (of which 6 are VUS-favor pathogenic), 22 LB, and 11 benign (Table 2). The largest shift in variant classification following reevaluation was in the VUS category, where 26 of 90 (29%) of the original VUSs were reclassified as LB or benign (Table 2). The next largest change in classification occurred in variants previously designated as LP, with 4 being downgraded to VUS-favor pathogenic and 1 being reclassified as pathogenic. No VUS, LB, or benign variants were advanced to a higher-grade category upon reclassification. These changes in variant classification led to a decrease in the number of “inconclusive” test results with VUS (60%–52%), and an increase in the proportion of “negative” tests that identified only LB or benign variants (14%–24%) (Table S2).

Familial Testing of Variants

Familial testing for 9 probands with a pathogenic or LP variant revealed that 3 variants were inherited and 6 variants were de novo mutations (Table 3). Additionally, 6 probands with a potentially disease-causing VUS underwent familial testing to help further clarify the variant's clinical significance. Of these 6 VUSs, 3 occurred de novo, and 3 were inherited from individuals without a cardiac structural phenotype at the time of testing.

Table 1. Clinical Characteristics of DCM Probands Who Underwent Genetic Testing

Patient Group	All	Age <1 year			Age >1 year			P Value [†]	
		All	+ Causal Variant*	- Causal Variant	P Value	All	+ Causal Variant*		- Causal Variant
No. of patients	63	23	8	15		40	11	29	
Age at presentation, y, median (range)	6.5 (0–22.3)	0.2 (0–0.9)	0.2 (0–0.9)	0.3 (0–0.8)	0.86	13.4 (1.3–22.3)	15.4 (5.5–18.3)	12.8 (1.3–22.3)	0.03
Male, n (%)	37/63 (59)	13/23 (57)	3/8 (38)	10/15 (67)	0.22	24/40 (60)	6/11 (55)	18/29 (62)	1
Positive family history, n (%)	11/60 (18)	0/23 (0)	0/8 (0)	0/15 (0)	NA	11/37 (30)	3/10 (30)	8/27 (30)	1
LVEF, median % (range)	26 (8–65)	23 (10–48)	23.5 (15–35.6)	19.4 (10–48)	0.45	29.5 (8–65)	30 (8–65)	29 (10–54)	0.46
LVEDV z score, median (range)	+5.85 (–0.1 to +21.1)	+5.5 (–0.1 to +21.1)	+4.7 (+2.6 to +9.3)	+6.65 (–0.1 to +21.1)	0.23	+6.3 (+1.6 to +24.2)	+6.3 (+1.7 to +13.4)	+6.3 (+1.6 to +24.2)	0.72
Follow-up time, y, median (range)	3.1 (0–9.8)	3.6 (0–9.7)	6.1 (0.8–9.7)	2.1 (0–9.4)	0.05	3.1 (0–9.8)	2.4 (0.1–9.3)	4.1 (0–9.8)	0.74
Death or transplant, n (%)	29/63 (46)	6/23 (26)	1/8 (13)	5/15 (33)	0.37	23/40 (58)	10/11 (91)	13/29 (45)	0.01

LVEDV indicates left ventricular end-diastolic volume; and LVEF, left ventricular ejection fraction.

*Includes likely pathogenic and pathogenic variants as per American College of Medical Genetics and Genomics and the Association for Molecular Pathology classification criteria, as well as those classified as variants of uncertain significance–favor pathogenic where clinical judgment was used to override the initial variant classification following clinical assessment.

[†]P value comparing age <1 year and age >1 year groups.

Table 2. Variant Classifications

Variants	After Reassessment						
		P	LP	VUS	LB	B	Not Categorized
Original	P	6*	2	0	0	0	0
	LP	1	6*	4 [†]	0	0	0
	VUS	0	0	64* [‡]	18	8	0
	LB	0	0	0	2*	1	0
	B	0	0	0	0	2*	0
	Not categorized	0	0	0	2	0	0

Variant classifications at the time of original testing and reclassification after applying current American College of Medical Genetics and Genomics and the Association for Molecular Pathology variant classification criteria. B indicates benign; LB, likely benign; LP, likely pathogenic; P, pathogenic; and VUS, variant of uncertain significance.

*Variants that did not change and shaded boxes indicate variant reclassifications.

[†]Four variants reclassified as VUS-favor pathogenic.

[‡]Two VUS-favor pathogenic.

Identification of Disease-Causing Variants

Pathogenic variants, LP, and VUS-favoring pathogenic, were identified in 12 genes, including those integral to sarcomere function (*MYH7*, *TTN*, *TNNT2*, *TNNI3*, *MYBPC3*, *TPM1*, *BAG3*), cellular structure (*LMNA*, *DES*), cellular junctions (*PKP2*, *DSP*), and lysosomal function (*LAMP2*) (Table 3). Nearly all mutations were heterozygous and thus demonstrated an autosomal dominant pattern. One proband was found to be hemizygous for a truncating mutation on the X chromosome encoded *LAMP2*. Nine de novo variants were identified in the following genes: *LAMP2*, *MYH7* (n=3), *LMNA*, *TNNI3*, *TPM1*, and *TNNT2* (n=2). Three of these variants in *MYH7*, *TNNT2*, and *TPM1* were classified as VUS-favor pathogenic, and within the clinical context were interpreted as causal in their respective cases. No individuals had a LP or pathogenic variant or a VUS-favor pathogenic variant in >1 gene. Collectively, 19 of 63 (30%) patients with idiopathic or familial DCM were determined to have a disease-causing variant, with at least 9 of 19 (47%) variants occurring de novo (Table 3). The most frequently identified mutations were in the sarcomeric genes *TNNT2* (n=5) and *MYH7* (n=3). All patients with mutations in *MYH7* presented in infancy, while those with *TNNT2* mutations presented at 2 discrete time points, either in early infancy (n=2) or adolescence (n=3). Rates at which disease-causing mutations were identified were similar between patients with a positive family history (3/11; 27%) and those without a positive family history (16/49; 33%). Additionally, the rate of detection of a causal variant did not differ by patient age (Table 1). The percentage of “positive” genetic tests that yielded a pathogenic or LP variant increased by 1.3-fold with expansion of the gene testing panel to >50 genes (20% for panels <50 genes versus 27% for >50 genes tested) and was largely unaffected by variant reclassification (Tables S2 and S3).

Outcomes of Patients With DCM

Patients with DCM who underwent genetic testing had a median clinical follow-up of 37 months (range, 0–118), and 29 of 63 (46%) patients underwent heart transplantation or died during follow-up. Echocardiographic measurements of left ventricular size and systolic function did not differ on the basis of the presence or absence of a causal variant in age-stratified patients (Table 1). Patients presenting after 1 year of age with a disease-causing variant had decreased transplant-free survival, with 10 of 11 (91%) patients having a composite outcome of death or transplant versus 13 of 29 (45%) patients >1 year of age without an identified variant ($P=0.01$) (Table 1 and Figure A). On proportional hazards modeling there was an increased risk of death or transplantation in patients presenting after the first year of life (hazard ratio [HR], 1.2; 95% CI, 1.05–1.3; $P=0.007$) (Figure B), consistent with previously reported age-related risk of death or transplantation.⁴ The presence of a disease-causing variant was not significantly associated with risk of transplantation or death in the overall cohort (HR, 1.9; 95% CI, 0.87–4.2; $P=0.11$) (Figure B). Transplant-censored survival was not different between groups, and the observed differences were largely driven by rates of heart transplantation (Figure C).

DISCUSSION

In this study, we performed a retrospective analysis of cardiomyopathy genetic testing in one of the largest reported pediatric cohorts with primary DCM to undergo genetic evaluation, and identified a disease-causing variant in 30% of patients. This detection rate for causal variants in probands was similar to results from adult DCM studies and other pediatric cohorts.^{9,15,17,18}

Table 3. Disease-Causing Variants

Genes	Transcript	Mutation	Mutation type	AA change	Classification	Age, y	Sex	Additional Clinical Data
<i>BAG3</i>	NM_004281.3	c.1363G>A	Missense	p.Glu455Lys	P	18.3	M	No familial data available
<i>DES</i>	NM_001927.3	c.347A>G	Missense	p.Asn116Ser	LP	11.5	M	No familial data available
<i>DSP</i>	NM_004415.2	c.1873C>T	Nonsense	p.Gln625X	P	16.0	F	Variant paternally inherited. Father (40 y) found to have DCM on cascade screening.
<i>LAMP2</i>	NM_002294.2	c.294G>A	Nonsense	p.Trp98X	P	16.7	M	De novo
<i>LMNA</i>	NM_170707.2	c.1106T>C	Missense	p.Leu369Pro	LP	12.7	F	De novo
<i>LMNA</i>	NM_170707.2	c.1621C>T	Missense	p.Arg541Cys	P	14.8	M	Mother (no genetic testing) with history of arrhythmia at 16 y and status post ICD placement. Sibling with variant and DCM phenotype at 12 years of age.
<i>MYBPC3</i>	NM_000256.3	c.2504delG	Frameshift	p.Arg835ProfsX2	P	0.9	M	Paternally inherited. Father (40 y) phenotype negative. Compound heterozygote with additional maternally inherited VUS in <i>MYBPC3</i>
<i>MYH7</i>	NM_000257.2	c.1106G>A	Missense	p.Arg369Gln	P	0.1	M	De novo
<i>MYH7</i>	NM_000257.2	c.1922G>C	Missense	p.Gly641Ala	LP	0.6	F	De novo
<i>MYH7</i>	NM_000257.2	c.1798C>T	Missense	p.Pro600Ser	VUS favor pathogenic	0.3	F	De novo
<i>PKP2</i>	NM_004572.3	c.1034+1G>T	Splicing	NA	LP	16.3	M	No familial data available
<i>TNNI3</i>	NM_000363.4	c.544G>A	Missense	p.Glu182Lys	LP	0.1	F	De novo
<i>TNNT2</i>	NM_001001430.1	c.629_631delAGA	Deletion/in-frame	p.Lys210del	P	14.4	F	Sibling with history of DCM and heart transplant at 12 y (genotype unavailable). Parental genotype/phenotype not available.
<i>TNNT2</i>	NM_001001430.1	c.629_631delAGA	Deletion/in-frame	p.Lys210del	P	16.6	M	De novo
<i>TNNT2</i>	NM_001001430.1	c.517C>T	Missense	p.Arg173Trp	LP	17.7	F	Two siblings genotype and phenotype negative, parental testing not available
<i>TNNT2</i>	NM_001001430.1	c.391C>T	Missense	p.Arg131Trp	LP	0.1	M	No familial data available
<i>TNNT2</i>	NM_001001430.1	c.264T>G	Missense	p.Asp88Glu	VUS favor pathogenic	0.3	F	De novo
<i>TPM1</i>	NM_000366.5	c.423G>C	Missense	p.Met141Ile	VUS favor pathogenic	0.1	F	De novo
<i>TTN</i>	NM_133378.4	c.65683delG	Frameshift	p.Ala21895ProfsX8	LP	14.0	F	Father (35 y) diagnosed with DCM on cascade screening. Genotype unknown.

Variants identified to be disease causing in the pediatric DCM cohort on the basis of pathogenic or likely pathogenic classification per American College of Medical Genetics and Genomics and the Association for Molecular Pathology classification criteria, as well as variants initially classified as VUS-favor pathogenic that were determined to be disease causing at the discretion of the clinical geneticist/cardiologist. Listed age is age at presentation.

ICD indicates implantable cardioverter defibrillator; LP, likely pathogenic; P, pathogenic; and VUS, variant of uncertain significance.

In probands presenting after 1 year of age, we observed lower transplant-free survival in those with a positive genetic testing result compared with those without an identified causal variant. Previous studies have similarly reported an association between genetic testing results and transplant-free survival.¹⁷ We are unable to report whether the natural history of patients with DCM with a positive genetic testing result differs significantly from those without an identified causal variant because of the high rates of transplantation

in our pediatric DCM population. As a result, the observed difference in transplant-free survival could be the result of confounding by indication.

Surprisingly, we did not find a difference in rates of positive cardiomyopathy genetic testing in patients with (27%) and without (33%) a family history of cardiomyopathy or sudden death. In our study, this is likely the result of a high proportion of de novo variants identified in patients with a negative family history. Few published case series have comprehensively evaluated

the mechanism of inheritance of variants in pediatric DCM, but small studies of pediatric DCM have shown a high fraction of de novo variants in established DCM genes.²⁶ In the 73% of patients with DCM and positive family history who did not have a causal variant identified, incomplete testing for the full spectrum of potential cardiomyopathy-associated genes, oligogenic or polygenic causes of disease, or undetected types of genomic variation such as structural variants could contribute to inability to identify a genetic cause. The lack of association between family history and genotype in our study supports the current guideline recommendation for clinical genetic testing in all cardiomyopathy probands, regardless of family history¹⁹ and the need for additional genetic studies in large cohorts of pediatric patients with DCM.

Our results highlight the importance of genetic and phenotypic evaluation in parents and first-degree

relatives of probands for the interpretation of genetic testing results in DCM probands. Familial testing of healthy parents provided supporting evidence of variant pathogenicity in 3 probands by identifying the variant as de novo. In our small cohort, the use of this familial data aided in the identification of 3 of 19 (16%) of the disease-causing variants. Given the large number of patients with VUSs and inconclusive test results in our data set, additional information ascertained from familial genetic testing and cardiac screening, particularly in the parents, could provide evidence to help interpret the clinical significance of these variants. Beyond the value of familial screening to interpretation of variants identified in probands, data from screening help inform risk stratification of siblings and family planning.

We report that variant reclassification was able to reduce the overall burden of VUSs by nearly 30%. This is primarily attributable to the availability of reference

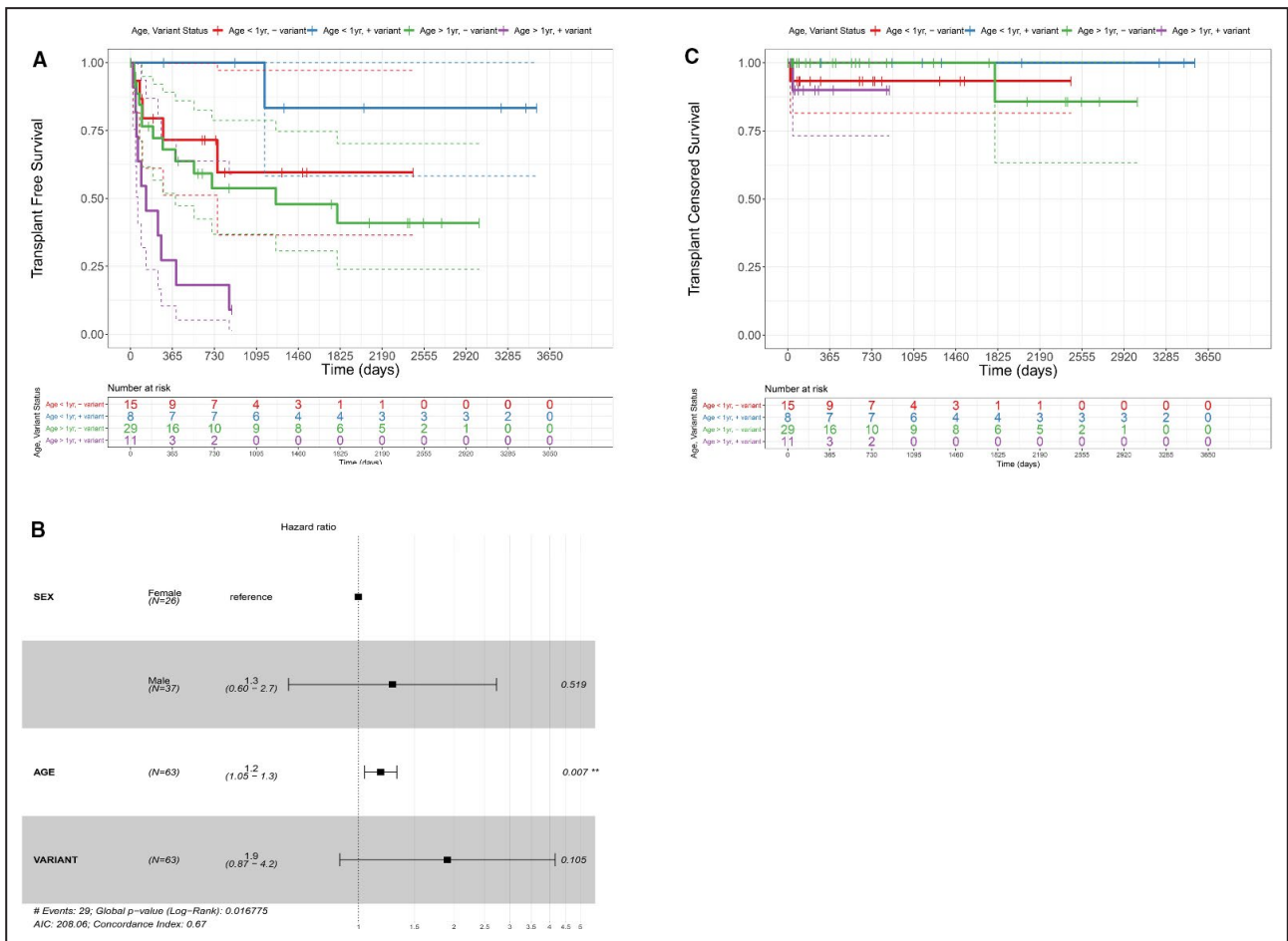


Figure. Kaplan–Meier analysis of transplant-free and transplant-censored Survival.

A, Kaplan-Meier survival analysis of transplant-free survival stratified by age and genetic testing. Patients who presented with DCM at >1 year of age and who had an identified causal genetic variant have significantly worse transplant-free survival when compared with those of a similar age without an identified genetic variant ($P=0.0139$, log-rank test). Dashed lines denote the 95% confidence interval. **B**, Kaplan-Meier survival analysis of transplant-censored survival demonstrates that differences in **(A)** are largely driven by the rate of transplantation. **C**, Forest plot of multivariate Cox proportional hazards modeling for transplant-free survival and selected covariates. Age but not DCM variant status is an independent risk factor for death or transplant. DCM indicates dilated cardiomyopathy.

databases such as gnomAD,²³ which allow the use of population allele frequencies to aid in the classification variants.^{23,27} Initial comparison of clinical genetic testing results in DCM with allele frequencies in the ExAC reference database demonstrated a large number of rare variants in purported DCM genes, leading to likely overestimation of pathogenicity in some cases.²⁸ Our results are in line with these findings and others,¹¹ with a large proportion of variants being downgraded on reclassification. Despite a reduction in the number of VUSs after reclassification, a large number of VUSs remain in our cohort. Additional population-level allele data that include diverse ethnic subgroups, increased data sharing among testing laboratories, and efforts to functionally classify VUSs will likely aid in further decreasing this burden. These results highlight the need for periodic reevaluation of variants, either by the testing laboratory, a geneticist, or cardiologist versed in variant interpretation. While general guidelines for variant reevaluation by testing laboratories have been put forth by the ACMG²⁹, there are no guidelines to inform when a clinician should initiate this process. A reasonable approach might be to perform a review of genetic testing if there is an inherited VUS or LP variant, more than a year has passed, and the parents are planning to have more children; when pediatric patients transition care to adult providers; or when discussing family planning with patients with primary DCM.

The disease-causing variants identified in our study are located in 12 genes that have been previously implicated in pediatric DCM. Variants in *MYH7* and *TNNT2* represent 25% of the disease-causing variants in our cohort and have been frequently identified in other pediatric DCM cohorts.^{8,9,11,17,30} All patients with a pathogenic *MYH7* variant presented in infancy, while the patients with *TNNT2* variants presented at 2 disparate time points, with 2 patients presenting in early infancy and 3 patients during adolescence. Three patients were found to have pathogenic variants in genes that are associated with arrhythmogenic cardiomyopathy (*DSP*, *PKP2*, and *DES*).³¹ Eight probands who presented in adolescence and in whom a pathogenic or LP variant was not identified were not tested for variants in *TTN*, the most frequently identified gene harboring pathogenic variants in DCM in both adolescent and adult patients.¹⁰ Given the importance of *TTN* variants in the pathogenesis of DCM and the increase in diagnostic yield observed with expansion of testing panels,¹¹ patients evaluated early in the era of genetic testing for DCM are likely to benefit from additional testing. As genetic testing costs decrease, a comprehensive whole genome sequencing approach may afford the best opportunity to identify pathogenic single-nucleotide variants, copy number variants, and/or polygenic causes of DCM.

There are several important limitations to our study. First, the use of diagnostic codes to identify DCM patients may not have identified all cases and may select for a subset with a more severe phenotype. All genetic testing was performed in the same laboratory, but there were significant changes in the number of cardiomyopathy-associated genes tested over the study period. As such, our study may underestimate the number of pathogenic or LP variants in this cohort, and those determined to have negative testing results early in the study period could harbor a pathogenic variant in untested genes. Finally, our data collection was retrospective, and some patients were lost to follow-up, which could underestimate the number of important clinical events.

Our work highlights that genetic testing of probands, especially when coupled with familial testing of first-degree relatives, is a useful diagnostic tool for identifying disease-causing variants and determining an etiology in patients with primary DCM. Genetic testing results are dynamic, and changes in variant classification over time significantly reduced the burden of VUSs; however, a significant number of probands with inconclusive test results remain. Additional genetic studies in this patient subgroup, continuous improvement of tools and data sharing mechanisms that aid in the clarification of the clinical significance of VUSs, and functional assays to better understand the effects of genetic variants on cardiomyocyte function will provide further clarification of VUSs. Additionally, a whole-genome sequencing approach to allow for the simultaneous evaluation of copy number variants and comprehensive gene sequencing in a significantly larger number of interrogated genes is likely to be the next frontier in the genetic evaluation of pediatric DCM.

ARTICLE INFORMATION

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Disclosures

None.

Supplementary Materials

Tables S1–S3

Figure S1

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SUPPLEMENTAL MATERIAL

Table S1. Cardiomyopathy gene testing panels.

Panel	DCM-pnlA	DCM-pnlB	PCM-pnlD	DCM-pnlC	PCM-pnlCv2	PCM-pnlA	PCM-pnlAv2	PCM-pnlCv3	PCM-pnlAv3
Genes	MYBPC3	LDB3	CASQ2	ABCC9	ABCC9	ABCC9	ABCC9	ABCC9	ABCC9
	MYH7	LMNA	DSC2	ACTC1	ACTC1	ACTC1	ACTC1	ACTC1	ACTC1
	TNNI3	ACTC1	DSG2	ACTN2	ACTN2	ACTN2	ACTN2	ACTN2	ACTN2
	TNNT2	PLN	DSP	CSRP3	BAG3	ANKRD1	ANKRD1	BAG3	ANKRD1
	TPM1	TAZ	JUP	CTF1	CSRP3	CASQ2	BAG3	CASQ2	BAG3
			PKP2	DES	CTF1	CAV3	CASQ2	CHRM2	CASQ2
			RYR2	EMD	DES	CRYAB	CAV3	CRYAB	CAV3
			TMEM43	LDB3	EMD	CSRP3	CRYAB	CSRP3	CHRM2
				LMNA	GATAD1	CTF1	CSRP3	DES	CRYAB
				MYBPC3	LAMP2	DES	CTF1	DMD	CSRP3
				MYH7	LDB3	DSC2	DES	DOLK	DES
				PLN	LMNA	DSG2	DSC2	DSC2	DMD
				SGCD	MYBPC3	DSP	DSG2	DSG2	DOLK
				TAZ	MYH7	DTNA	DSP	DSP	DSC2
				TCAP	NEXN	EMD	DTNA	DTNA	DSG2
				TNNI3	PLN	FHL2	EMD	EMD	DSP
				TNNT2	RBM20	GLA	FHL2	GATAD1	DTNA
				TPM1	SCN5A	JUP	GATAD1	GLA	EMD
				VCL	SGCD	LAMA4	GLA	JUP	FHL2
					TAZ	LAMP2	JUP	LAMP2	GATAD1
					TCAP	LDB3	LAMA4	LDB3	GLA
					TNNC1	LMNA	LAMP2	LMNA	ILK
					TNNI3	MYBPC3	LDB3	MURC	JPH2
					TNNT2	MYH6	LMNA	MYBPC3	JUP
					TPM1	MYH7	MYBPC3	MYH6	LAMA4
					TTN	MYL2	MYH6	MYH7	LAMP2
					VCL	MYL3	MYH7	MYL2	LDB3
						MYLK2	MYL2	MYL3	LMNA
						MYOZ2	MYL3	MYOZ2	MURC
						NEXN	MYLK2	MYPN	MYBPC3
						PKP2	MYOZ2	NEBL	MYH6
						PLN	NEBL	NEXN	MYH7
						PRKAG2	NEXN	PKP2	MYL2
						RBM20	PKP2	PLN	MYL3
						RYR2	PLN	PRDM16	MYLK2
						SGCD	PRKAG2	PRKAG2	MYOM1
						TAZ	RBM20	PTPN11	MYOZ2
						TCAP	RYR2	RAF1	MYPN
						TMEM43	SCN5A	RBM20	NEBL
						TNNC1	SGCD	RYR2	NEXN

						<i>TNNI3</i>	<i>TAZ</i>	<i>SCN5A</i>	<i>PDLIM3</i>
						<i>TNNT2</i>	<i>TCAP</i>	<i>SGCD</i>	<i>PKP2</i>
						<i>TPM1</i>	<i>TMEM43</i>	<i>TAZ</i>	<i>PLN</i>
						<i>TTN</i>	<i>TMPO</i>	<i>TCAP</i>	<i>PRDM16</i>
						<i>TTR</i>	<i>TNNC1</i>	<i>TMEM43</i>	<i>PRKAG2</i>
						<i>VCL</i>	<i>TNNI3</i>	<i>TNNC1</i>	<i>PTPN11</i>
							<i>TNNT2</i>	<i>TNNI3</i>	<i>RAF1</i>
							<i>TPM1</i>	<i>TNNT2</i>	<i>RBM20</i>
							<i>TTN</i>	<i>TPM1</i>	<i>RYR2</i>
							<i>TTR</i>	<i>TRDN</i>	<i>SCN5A</i>
							<i>VCL</i>	<i>TTN</i>	<i>SGCD</i>
								<i>TTR</i>	<i>TAZ</i>
								<i>VCL</i>	<i>TCAP</i>
									<i>TMEM43</i>
									<i>TNNC1</i>
									<i>TNNI3</i>
									<i>TNNT2</i>
									<i>TPM1</i>
									<i>TRDN</i>
									<i>TTN</i>
									<i>TTR</i>
									<i>VCL</i>

Genes included in cardiomyopathy sequencing panels. Testing panels expanded from 5 to 62 genes over the study period.

Table S2. Relationship between number of genes tested and test result.

Test Result		Positive		Inconclusive		Negative	
Re-classification status		Before	After	Before	After	Before	After
Number of genes tested	<= 10	1/6	3/6	3/6	1/6	2/6	1/6
	> 10 - 50	4/24	3/24	13/24	11/24	7/24	10/24
	> 50	11/33	9/33	22/33	20/33	0/33	4/33
	All	16/63	15/63	38/63	32/63	9/63	15/63

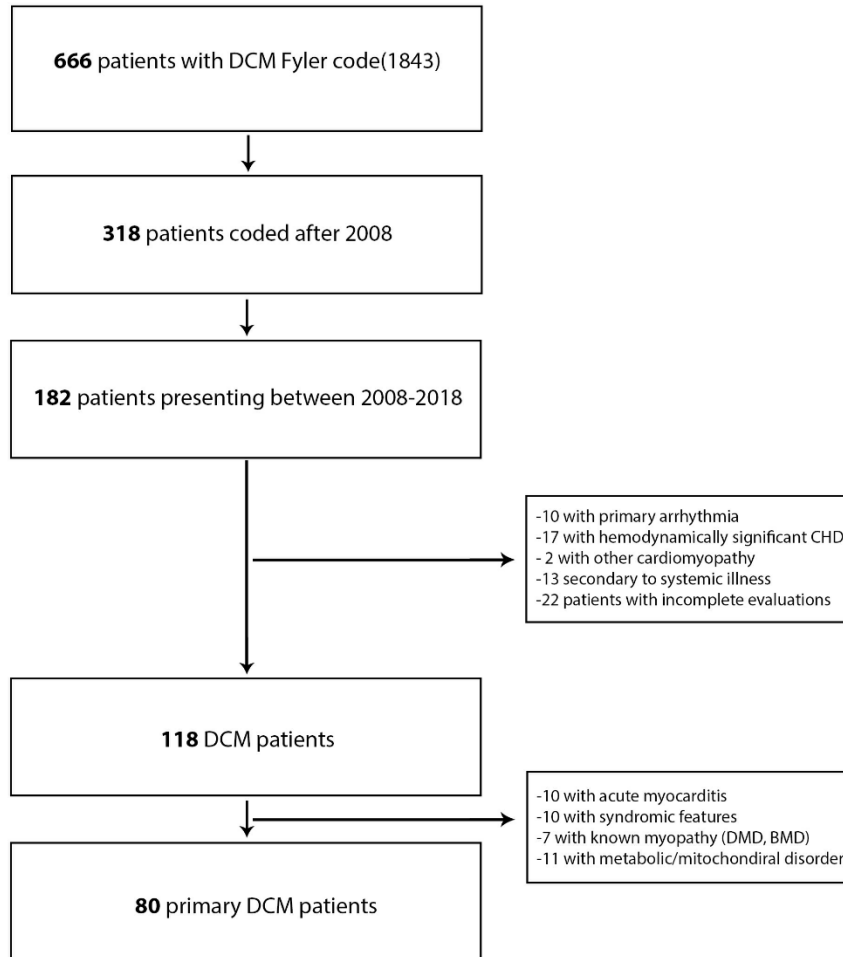
Test results before and after variant reclassification as a proportion of the total number of tests are shown. Tests are grouped by number of genes tested. Positive tests include those with pathogenic or likely pathogenic variants. Negative tests include those with benign or likely benign variants. Inconclusive tests include those with VUS's that do not meet positive test criteria. Variants that were ultimately determined to be disease causing at the discretion of the clinician are grouped with their original testing result as issued by the laboratory.

Table S3. Number of variants identified by genetic testing panel.

Panel(s) Tested	DCM- pnlA	DCM- pnlB	PCM- pnlD	DCM- pnlA, DCM- pnlB	DCM- pnlC	PCM- pnlCv2	PCM- pnlA	PCM- pnlAv2	PCM- pnlCv3	PCM- pnlAv3
Number of genes tested	5	5	8	10	19	27	46	51	53	62
Number of tests performed	1	1	1	3	18	5	1	9	1	23
Number of causal variants* identified	0	1	0	2	5	1	0	4	0	6

Genetic testing by gene panel. A list of genes for each panel can be found in Table S2. *includes Likely pathogenic and pathogenic variants as per ACMG/AMP classification criteria, as well as those classified as VUS favor pathogenic where clinical judgement was used to override the initial variant classification following clinical assessment

Figure S1. Patient selection scheme.



80 patients with primary DCM were identified within the study period. 73 patients were probands without prior genetic evaluations, and 63 probands that underwent genetic testing included in this study.