Genetic causes of cardiomyopathy in children: First results from the Pediatric Cardiomyopathy Genes Study

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et al

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ORIGINAL RESEARCH

Genetic Causes of Cardiomyopathy in Children: First Results From the Pediatric Cardiomyopathy Genes Study

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BACKGROUND: Pediatric cardiomyopathy is a genetically heterogeneous disease with substantial morbidity and mortality. Current guidelines recommend genetic testing in children with hypertrophic, dilated, or restrictive cardiomyopathy, but practice variations exist. Robust data on clinical testing practices and diagnostic yield in children are lacking. This study aimed to identify the genetic causes of cardiomyopathy in children and to investigate clinical genetic testing practices.

METHODS AND RESULTS: Children with familial or idiopathic cardiomyopathy were enrolled from 14 institutions in North America. Probands underwent exome sequencing. Rare sequence variants in 37 known cardiomyopathy genes were assessed for pathogenicity using consensus clinical interpretation guidelines. Of the 152 enrolled probands, 41% had a family history of cardiomyopathy. Of 81 (53%) who had undergone clinical genetic testing for cardiomyopathy before enrollment, 39 (48%) had a positive result. Genetic testing rates varied from 0% to 97% between sites. A positive family history and hypertrophic cardiomyopathy subtype were associated with increased likelihood of genetic testing ($P=0.005$ and $P=0.03$, respectively). A molecular cause was identified in an additional 21% of the 63 children who did not undergo clinical testing, with positive results identified in both familial and idiopathic cases and across all phenotypic subtypes.

CONCLUSIONS: A definitive molecular genetic diagnosis can be made in a substantial proportion of children for whom the cause and heritable nature of their cardiomyopathy was previously unknown. Practice variations in genetic testing are great and should be reduced. Improvements can be made in comprehensive cardiac screening and predictive genetic testing in first-degree relatives. Overall, our results support use of routine genetic testing in cases of both familial and idiopathic cardiomyopathy.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT01873963.

**Key Words:** exome ■ heart failure ■ infant ■ molecular ■ mutation

Cardiomyopathy is a clinically and genetically heterogeneous form of heart muscle disease with substantial morbidity and mortality in children. There are 5 phenotypes: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), left ventricular noncompaction...
Cardiovascular disease is a leading cause of death worldwide and includes conditions such as coronary artery disease, hypertension, heart failure, and congenital heart defects. Several recent meta-analyses and reviews have highlighted the role of genetic factors in cardiovascular disease, suggesting that genetic testing and counseling for cardiomyopathy are indicated. However, the current guidelines recommend genetic testing for cardiomyopathy in children and adults with familial or idiopathic cardiomyopathy. The purpose of this study was to determine the prevalence of pathogenic variants in known cardiomyopathy genes and to identify practice variation in the genetic evaluation of children with cardiomyopathy.

**Clinical Perspective**

**What Is New?**
- There is significant practice variation in ordering genetic testing for pediatric patients with cardiomyopathy.
- In a pediatric cardiomyopathy cohort in which =50% had clinical genetic testing, 21% of non-tested patients had a diagnostic genetic finding with research-based testing of known cardiomyopathy genes.

**What Are the Clinical Implications?**
- Routine use of genetic testing in children with familial or idiopathic cardiomyopathy is indicated.

**Nonstandard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>DCM</td>
<td>Dilated cardiomyopathy</td>
</tr>
<tr>
<td>HCM</td>
<td>Hypertrophic cardiomyopathy</td>
</tr>
<tr>
<td>LVNC</td>
<td>Left ventricular noncompaction</td>
</tr>
<tr>
<td>PCMR</td>
<td>Pediatric cardiomyopathy registry</td>
</tr>
<tr>
<td>RCM</td>
<td>Restrictive cardiomyopathy</td>
</tr>
<tr>
<td>VUS</td>
<td>Variant of uncertain significance</td>
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</table>

Cardiovascular disease is a leading cause of death worldwide and includes conditions such as coronary artery disease, hypertension, heart failure, and congenital heart defects. Several recent meta-analyses and reviews have highlighted the role of genetic factors in cardiovascular disease, suggesting that genetic testing and counseling for cardiomyopathy are indicated. However, the current guidelines recommend genetic testing for cardiomyopathy in children and adults with familial or idiopathic cardiomyopathy. The purpose of this study was to determine the prevalence of pathogenic variants in known cardiomyopathy genes and to identify practice variation in the genetic evaluation of children with cardiomyopathy.

**Methods**

**Study Design**

The study used the network established by the PCMR (Pediatric Cardiomyopathy Registry) for exome sequencing in a large prospectively recruited cohort. The overall goals of the study are to develop approaches to exome analysis for autosomal dominant disease, to determine genotype-phenotype correlations and to identify genetic modifiers that influence the long-term clinical course of children with cardiomyopathy. Here, we present the first results of the study, in which we determine the prevalence of pathogenic variants in known cardiomyopathy genes; investigate the associations between phenotype and age of onset, sex, race, and ethnic correlates; and identify practice variation in genetic testing of these children.
University (2014–2018), and the University at Buffalo (2018–2020) served as the Administrative Coordinating Centers, which were responsible for regulatory and clinical adherence, study implementation, protocol guidance, data collection (Appendix S1), and analysis planning. The New England Research Institute was the Data and Statistical Coordinating Center. Cincinnati Children’s Hospital Medical Center (2012–2014) and Indiana University (2014–2020) served as Genetic Coordinating Centers. All analytic and study materials are available within this article and its online supplementary files. Deidentified genomic data will be publicly available within the database of Genotypes and Phenotypes.

Patients
Enrollment for the study began April 12, 2013 and continued through February 29, 2016. Children were eligible if they had a diagnosis of idiopathic or primary cardiomyopathy with a phenotype of DCM, HCM, RCM, or LVNC or presumed myocarditis before 18 years of age. Diagnoses were ascertained by chart review and confirmed by echocardiography (Table S1) or cardiac magnetic resonance imaging. Children with cardiomyopathy secondary to another condition such as neuromuscular disease or a genetic syndrome associated with cardiomyopathy were excluded (Table S2).

Biological parents and affected siblings or relatives were approached for enrollment. After written informed consent or assent, blood was drawn for genetic testing and a 3-generation pedigree was obtained. Saliva samples were collected when blood collection failed. A positive family history was defined as at least 1 additional biological family member with a diagnosis of cardiomyopathy or with positive genetic testing results for cardiomyopathy if phenotype negative. If clinical genetic testing was already performed, results from the testing laboratory were uploaded to the database. All clinical genetic results were manually reviewed.

For this report, clinical genetic testing refers to molecular testing for cardiomyopathy, typically with a next-generation sequencing cardiomyopathy panel; other types of clinical genetic testing, such as chromosomes, were excluded.

Cardiac phenotypic data were collected for 3 years before enrollment until 2 years after unless the child died, underwent heart transplant, or withdrew from the study. Data collected at study visits included demographic information, anthropometric measurements, family history with pedigree, eligibility data, heart failure class, the results of cardiac studies (echocardiograms, cardiac magnetic resonance imaging, ECGs, Holter monitoring, and endomyocardial biopsies), hospitalizations, cardiac transplant status, and cause of death. Study data were entered into a web-based eClinicalOS system through dedicated, password-protected study computers. The Institutional Review Boards at the Administrative Coordinating Center and participating institutions approved this study.

Sample Collection, Preparation, and Sequencing
Blood or saliva samples were collected at each site and shipped to the Genetic Coordinating Center. DNA was extracted on a Maxwell RSC instrument (Promega, Madison, WI) according to the manufacturer’s protocol. Exome sequencing was performed using sequence capture (SeqCap EZ Human Exome 2.0; Nimblegen, Madison, WI) and a HiSeq2500 sequencer (Illumina, San Diego, CA) at Cincinnati Children’s Hospital Medical Center in a Clinical Laboratory Improvement Amendments–approved lab. All samples were sequenced to a mean coverage of 72× (36× to 148×) (Table S3). Alignment was performed to Genome Reference Consortium Human Build 37 GRCh37 with BWA aln/sampe version 0.5.9 using default parameters. Genome Analysis Toolkit version 3.3 best practices were followed for variant calling, and variants were annotated using a Cincinnati Children’s Hospital Medical Center in-house script, according to their predicted impact on University of California–Santa Cruz Known Genes (hg19).

Variant Assessment
At study initiation, 37 genes from clinical cardiomyopathy genetic testing panels were selected for interrogation (Table 1). These genes were selected at study initiation based on panels that were orderable from commercial clinical laboratories. In addition, they had been shown clinically or in research testing in children to cause disease. The one exception was the TTR gene, which is not associated with cardiomyopathy in children but was included as a potential modifier. Results were returned to participants for all genes (n=36) except TTR if return of results was elected upon consent. Among

Table 1.  Cardiomyopathy-Causing Genes Analyzed in the Pediatric Cardiomyopathy Genes Study

<table>
<thead>
<tr>
<th>ABC29</th>
<th>LAMP2</th>
<th>NEXN</th>
<th>TNNC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTG1</td>
<td>LDG3</td>
<td>PLN</td>
<td>TNNI3</td>
</tr>
<tr>
<td>ACTN2</td>
<td>LAMA</td>
<td>PKRAG2</td>
<td>TNNT2</td>
</tr>
<tr>
<td>ANKRD1</td>
<td>MYBP3</td>
<td>RBM20</td>
<td>TPM1</td>
</tr>
<tr>
<td>BAG3</td>
<td>MYH6</td>
<td>SCN5A</td>
<td>TTN</td>
</tr>
<tr>
<td>CAV3</td>
<td>MYH7</td>
<td>SCO2</td>
<td>TTR</td>
</tr>
<tr>
<td>CFYAB</td>
<td>MYL2</td>
<td>SGCD</td>
<td>VCL</td>
</tr>
<tr>
<td>CSRPR3</td>
<td>MYL3</td>
<td>SURF1</td>
<td></td>
</tr>
<tr>
<td>DES</td>
<td>MYPN</td>
<td>TAZ</td>
<td></td>
</tr>
<tr>
<td>EMD</td>
<td>NEBL</td>
<td>TCAP</td>
<td></td>
</tr>
</tbody>
</table>
152 children, 6 (4%) declined to receive genetic results. A positive result was defined as ≥1 pathogenic or likely pathogenic variants known to cause cardiomyopathy in children. Results that confirmed previous clinical testing results were not returned to participants; thus, only new positive findings were returned.

Filtering was performed (single-nucleotide polymorphism database 144, minor allele frequency <5%) at low stringency to retain potential modifying variants for future studies. Retained variants were predicted to alter the protein (missense, frameshift, insertion/deletion, stop gained or lost, and splice-site mutations). Because of the large number of variants in the TTN gene encoding the protein titin and good evidence for truncating variants in TTN causing DCM, only nonsense and frameshift variants located within the A-band region of the protein were interpreted.25,26

For all 37 genes, potential protein-altering rare variants were interpreted according to the American College of Medical Genetics and Genomics standards and guidelines for the interpretation of sequence variants to assess pathogenicity.27 (Table S4). All variant interpretations were concordant with ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) as of December 1, 2018. Variants were interpreted as pathogenic, likely pathogenic, VUS, likely benign, or benign (Table S4). All novel variants are being deposited into ClinVar.

**RESULTS**

**PCM Genes Participants**

Demographics for the first 152 probands enrolled in the PCM Genes, representing 9 of the 14 participating sites, are shown in Table 2. The cohort was 51% male and predominantly non-Hispanic White. Median age (interquartile range) at diagnosis was 4.5 (0.5–13.2) years (Table 2). Median age (interquartile range) at enrollment was 10.5 (3.3–15.7) years. The ratio of DCM to HCM was about 2:1, which is consistent with other epidemiologic studies.6,8,20,26-29 (Figure 1). Children with RCM represented 9% of the cohort, slightly higher than the reported frequency of 5% among all cardiomyopathies.30 Three children (2%) had isolated LVNC, and 13

Table 2. Characteristics of Children With Cardiomyopathy in the Pediatric Cardiomyopathy Genes Study, by Phenotype

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All, N=152</th>
<th>Type of Cardiomyopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dilated, n=80</td>
</tr>
<tr>
<td>Male, %</td>
<td>51.3</td>
<td>43.8</td>
</tr>
<tr>
<td>Race/ethnicity, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>73.7</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>13.2</td>
<td>16.3</td>
</tr>
<tr>
<td>East Asian, South Asian</td>
<td>13.2</td>
<td>17.5</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>81.6</td>
<td>83.8</td>
</tr>
<tr>
<td>Median (IQR) age at diagnosis, y</td>
<td>4.5 (0.5–13.2)</td>
<td>1.9 (0.3–11.1)</td>
</tr>
<tr>
<td>Median (IQR) age at enrollment, y</td>
<td>11.2 (4.8–16.8)</td>
<td>10.5 (3.3–15.7)</td>
</tr>
<tr>
<td>Median (IQR) time from diagnosis to enrollment, y</td>
<td>2.7 (0.5–6.9)</td>
<td>3.8 (0.6–7.6)</td>
</tr>
<tr>
<td>Family history, % positive</td>
<td>40.8</td>
<td>28.8</td>
</tr>
</tbody>
</table>

IQR indicates interquartile range.
(9%) had a mixed phenotype that frequently included LVNC (9/13; 69%).

**Differences by Cardiomyopathy Phenotype**

Age at diagnosis differed significantly between the cardiomyopathy phenotypes \((P<0.0001; \text{Table 2 and Figure 1})\). These differences were largely driven by the higher median age at diagnosis for HCM than for DCM \((P<0.0001)\) and LVNC/Mixed \((P=0.0004)\). The phenotypes also differed by sex \((P=0.02)\), with HCM having more males (71%) than the other phenotypes, and by race or ethnicity \((P=0.02)\), with HCM and RCM having higher proportions of non-Hispanic White individuals (81% and 71%, respectively) than DCM and LVNC/Mixed (58% and 44%, respectively). Children with HCM had the highest frequency of a positive family history (67%), with DCM and RCM each having 29% \((P<0.001)\).

**Clinical Genetic Testing Practices and Outcomes**

Figure 2 shows the proportion of patients who underwent clinical genetic testing. Overall, 89 children (59%) had results available at the time of exome analysis. Of these, 81 (53%) had a cardiomyopathy gene panel, and another 8 had other genetic testing (targeted testing for a known familial variant [6], exome testing [1], and unspecified cardiomyopathy genetic testing [1]). These 8 children were excluded when the frequency and yield of clinical panel testing was calculated (Figure 2).

We evaluated factors associated with cardiomyopathy gene panel testing between those who did and did not have previous clinical test results (Table 3). Children who had undergone testing had a higher rate of positive family history (51% versus 22%; \(P=0.005)\). Age of enrollment, sex, and race were not associated with clinical genetic testing, but cardiomyopathy phenotype was \((P=0.03; \text{Figure 2})\). Specifically, children with HCM were more likely than children with DCM to undergo clinical testing (74% versus 46%, \(P=0.005)\). In a logistic regression model, only family history retained significance \((\text{OR}=3.1 [1.4–6.8]; P=0.0044)\). The lack of significance for type of CM was likely attributable to the strong association between type of cardiomyopathy and family history with HCM having a much higher rate of positive family history than DCM (67% versus 29%,
respectively; \( P<0.0001 \)). Variation across the sites was marked (Figure S1). Of sites contributing at least 10 cases, 3 sites performed testing in less than one-third of children (range, 19%–32%), 1 site performed testing in about half (47%), and 3 sites performed testing in at least two-thirds of children (range, 68%–97%).

Of 81 children who underwent clinical cardiomyopathy gene panel testing, 39 (48%) had a positive result; 19 (24%) had a VUS as the primary finding; and 23 (28%) had negative genetic testing results (Figure 3). Overall, the frequency of positive results was higher in children with HCM (68%) than with DCM (31%; \( P=0.0008 \)).

### Sequencing Results

Of the variants reported for children who had already undergone clinical testing, 89% were also identified by exome sequencing, with indel variants in TTN accounting for most of the variants missed by exome. In the 63 children who had not previously undergone clinical genetic testing, a group predicted to have a lower diagnostic yield because of the increased number of children with DCM and children without a family history of disease, 21% (13/63) had an identifiable pathogenic or likely pathogenic variant. Four had DCM, 6 had HCM, 2 had RCM, and 1 had LVNC/Mixed. Of these 13 children, 12 had a single variant identified as pathogenic or likely pathogenic, and 1 child had 2 variants.

Based on exome sequencing of 37 known cardiomyopathy genes, 32% (49/152) had a positive pathogenic or likely pathogenic finding. Five children (3%) had 2 pathogenic or likely pathogenic variants; no child had >2. Positive results (Table S4) differed by cardiomyopathy type and family history (Figure 4). Diagnostic yields for familial DCM, HCM, RCM, and LVNC/Mixed cases were 35%, 68%, 75%, and 43%, respectively. Although idiopathic cases of HCM or RCM also had high diagnostic yields of 36% and 50%, respectively, idiopathic DCM cases tested positive less frequently (9%). None of the 3 children with isolated LVNC had positive findings, although 2 of the 3 had a positive family history of cardiomyopathy.

### Cardiac Surveillance and Genetic Testing in Family Members

Cardiac surveillance in first-degree relatives of a child with cardiomyopathy is recommended. Similarly, predictive genetic testing is indicated in first-degree relatives of those with a pathogenic or likely pathogenic variant. Among our patients with a positive family history of cardiomyopathy, 89% (55/72 familial cases) had

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**Figure 2.** Frequency of clinical genetic cardiomyopathy panel testing by cardiomyopathy subtype.

DCM indicates dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LVNC, left ventricular noncompaction; and RCM, restrictive cardiomyopathy.

**Figure 3.** Clinical genetic testing results in 81 cardiomyopathy patients by phenotype.

Results interpreted as pathogenic or likely pathogenic are considered positive. Patients who did not have cardiomyopathy panel testing were excluded. DCM indicates dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LVNC, left ventricular noncompaction; RCM, restrictive cardiomyopathy; and VUS, variant of uncertain significance.

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**Table 3. Factors Associated With Clinical Genetic Testing in Children With a Cardiomyopathy Diagnosis**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Clinical Genetic Testing</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes*, n=81</td>
<td>No, n=63</td>
</tr>
<tr>
<td>Median (IQR) age at diagnosis, y</td>
<td>5.5 (0.7–13.2)</td>
<td>2.9 (0.4–13.2)</td>
</tr>
<tr>
<td>Male, %</td>
<td>49.4</td>
<td>49.2</td>
</tr>
<tr>
<td>Race, non-Hispanic White, %</td>
<td>65.4</td>
<td>58.7</td>
</tr>
<tr>
<td>Family history, % positive</td>
<td>50.6</td>
<td>22.2</td>
</tr>
<tr>
<td>Cardiomyopathy type, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertrophic</td>
<td>34.8</td>
<td>15.9</td>
</tr>
<tr>
<td>Dilated</td>
<td>44.4</td>
<td>66.7</td>
</tr>
<tr>
<td>Restrictive</td>
<td>8.6</td>
<td>9.5</td>
</tr>
<tr>
<td>Left ventricular noncompaction/mixed</td>
<td>12.4</td>
<td>7.9</td>
</tr>
</tbody>
</table>

IQR indicates interquartile range.

*Eight children with genetic testing other than cardiomyopathy panel testing (eg, testing for known familial variant) were excluded.
cardiac surveillance in first-degree relatives, whereas in those without a family history, 42% (38/90 idiopathic cases) had cardiac surveillance of first-degree relatives. When focusing on the subset of probands who did not have clinical genetic testing (n=63), 10 of 14 familial cases (71%) had cardiac imaging in first-degree relatives, whereas 16 of 49 idiopathic cases (41%) had cardiac screening in first-degree relatives. Overall, predictive genetic testing was performed in 58% of first-degree relatives of familial cases. In probands with no documented family history who underwent clinical genetic testing, 3 had presumed de novo pathogenic variants (both parents documented as negative for the pathogenic variant), 4 had VUS results that were inherited from an unaffected parent, and 1 had clinical exome testing performed as a quad test with mother, father, and brother, which was negative. Of note, this study was not longitudinal in nature and therefore additional family members may have undergone cardiac screening or predictive genetic testing since study enrollment. Importantly, 77% (10/13) of children with positive exome results who had not previously had clinical testing also had no family history of disease. For the entire cohort, the 14 children with new positive molecular findings had 44 at-risk first-degree relatives (parents and siblings).

**DISCUSSION**

We conducted a multicenter study to better understand the genetic basis of familial and idiopathic pediatric cardiomyopathy. Information collected on 152 children provided important new data about clinical genetic testing practices in North America. With research sequencing on 152 children, we identified minimum diagnostic yields in known cardiomyopathy genes for broadly inclusive genetic testing in children with either familial or idiopathic cardiomyopathy.

Previous studies of the PCMR have provided information on the epidemiologic features and clinical outcomes of children with cardiomyopathy or heart failure, and an understanding of the causal categories and their impact on risk stratification and outcome, but information on genetic causes specifically in the pediatric population has been limited. The PCM Genes study was intended to establish disease-specific genotype-phenotype correlations in pediatric cardiomyopathy and to provide a more sophisticated understanding of the molecular basis of disease and its association with presentation, clinical course, and outcome.

Another PCMR study found that 51% of children had a diagnosis of DCM, a finding similar to the 53% (80/152) of our cohort. However, HCM (n=42) was less common in our cohort (28% versus 42%), and, as anticipated, this group had the highest proportion of children with positive family histories of cardiomyopathy. Somewhat surprisingly, the frequency of family history of cardiomyopathy in the LVNC/Mixed cardiomyopathy group was 44% (7/16). Two recent publications recommend genetic testing when HCM or DCM is seen in conjunction with LVNC and do not recommend testing for isolated LVNC in the absence of a family history. An additional publication of LVNC patients of all ages that included 52 patients <18 years of age also found a low genetic diagnostic yield in sporadic cases of isolated LVNC and determined that left ventricular systolic dysfunction at presentation and long-term outcome were related to genetics. All positive cases in our LVNC/Mixed group would have been identified clinically following these guidelines. However, with only 16 children, our LVNC/Mixed group was small, and additional studies are required to better understand the genetic basis of mixed cardiomyopathy phenotypes.

Our results show that although genetic testing is being widely used it is not yet universal, and variations in practice are great. The frequency of testing ranged from none to 97% of patients. In previous studies, factors influencing decisions to order or recommend genetic testing have included perceived ability to obtain testing, knowledge and comfort level with understanding and discussing results, and perception of benefits or clinical utility. At the time of study initiation, no clinical guidelines existed that specifically recommended genetic testing for cardiomyopathy in children, which may account for some of the variability. The availability and integration of geneticist and genetic counselor expertise as well as the cardiologists’ acceptance of the importance of cardiac genetics may also have played
roles, although this study was not designed to assess this directly. A recent study has shown that genetic testing is less used in index patients with HCM than in long QT syndrome.\(^4\) At-risk first-degree family members of a child with cardiomyopathy or long QT syndrome are more likely to participate in recommended cardiac screening than genetic testing,\(^5\) although screening participation was higher in families of gene-positive children. In the current study, family history status and cardiomyopathy type were significantly associated with performing testing. However, family history status is at least partially dependent on comprehensive cardiac surveillance in first-degree relatives. First-degree relatives underwent cardiac screening in 42% of idiopathic cases, potentially leading to an underestimation of the prevalence of familial disease in affected asymptomatic family members. In the field of cardiovascular genetics, barriers to cardiac screening or genetic testing centered on patient concerns have not been well studied, but at least 1 study suggests that family decisions are more frequently the cause than insurance.\(^4\) Further studies investigating motivation and barriers to genetic testing in children with cardiomyopathy will be necessary to delineate provider- and family-specific factors.

We identified pathogenic or likely pathogenic variants in 32% of children (49/152). Among tested patients, HCM and RCM both had a high diagnostic yield and a low frequency of VUS. Because RCM is quite rare, yields of genetic testing have not been widely reported, although a 2008 study previously reported 4 of 12 RCM patients with mutations in sarcomeric genes including ACTC, TNNT2, and TNNI3,\(^4\) although screening participation was higher in families of gene-positive children. In the current study, family history status and cardiomyopathy type were significantly associated with performing testing. However, family history status is at least partially dependent on comprehensive cardiac surveillance in first-degree relatives. First-degree relatives underwent cardiac screening in 42% of idiopathic cases, potentially leading to an underestimation of the prevalence of familial disease in affected asymptomatic family members. In the field of cardiovascular genetics, barriers to cardiac screening or genetic testing centered on patient concerns have not been well studied, but at least 1 study suggests that family decisions are more frequently the cause than insurance.\(^4\) Further studies investigating motivation and barriers to genetic testing in children with cardiomyopathy will be necessary to delineate provider- and family-specific factors.

We identified pathogenic or likely pathogenic variants in 32% of children (49/152). Among tested patients, HCM and RCM both had a high diagnostic yield and a low frequency of VUS. Because RCM is quite rare, yields of genetic testing have not been widely reported, although a 2008 study previously reported 4 of 12 RCM patients with mutations in sarcomeric genes including ACTC, TNNT2, and TNNI3.\(^4\) In this study, 8 of 14 (57%) had pathogenic or likely pathogenic variants identified, suggesting that more widespread use of testing in this population should be considered. In contrast, the positive and VUS frequencies were nearly equal in children with DCM and LVNC/Mixed phenotypes, perhaps reflecting a greater genetic heterogeneity in these subtypes. Of 63 children without previous genetic testing, positive results were identified in 13 (21%), as well as in 1 child whose previous test results were negative. These missed opportunities for a molecular diagnosis by clinical testing have important implications for first-degree relatives for whom targeted familial testing can be offered, allowing risk stratification and targeted cascade screening. Ongoing cardiac surveillance is indicated in first-degree relatives of a child with cardiomyopathy when the cause is unknown.\(^2-4\) For these 14 families with a new molecular diagnosis, testing for the known familial pathogenic variant in the 44 first-degree relatives can identify individuals who require ongoing surveillance and those who do not and is cost-effective by limiting ongoing evaluations and cardiac imaging to at-risk family members.\(^15,45\) Importantly, these missed genetic diagnoses occurred across all types of cardiomyopathy, indicating that all functional phenotypes of pediatric cardiomyopathy have a potential molecular cause identifiable through genetic testing.

A family history of cardiomyopathy indicates a genetic cause, and therefore it was not surprising to find a significant association with clinical genetic testing in this study. Interestingly, we also identified pathogenic genetic variants in 36% of children with idiopathic HCM and 50% of children with idiopathic RCM. Although DCM typically has a lower diagnostic yield than that in other types of cardiomyopathy (20%–30%), we found that 35% with familial DCM had positive results. In idiopathic DCM, the rate was lower (9%). Nevertheless, reclassifying 9% of idiopathic DCM cases as heritable substantially alters the care for family members. Thus, family history status may be useful to estimate prior probability of a positive genetic test but cannot be used to rule out the need for genetic testing. Overall, our results support use of genetic testing in cases of both familial and idiopathic cardiomyopathy.

**Strengths and Limitations of the Study**

Children were enrolled at centers with known expertise in pediatric cardiomyopathy; therefore, we cannot exclude ascertainment bias for more severe or atypical cases. All eligible patients with cardiomyopathy were approached, but we cannot completely eliminate the possibility of a survivor bias. This study was not longitudinal in nature and family history is dynamic. Cardiac surveillance and predictive cascade clinical genetic testing in family members may have occurred on a clinical basis after study data capture. Because we tested for 37 known cardiomyopathy genes, the diagnostic yield for each phenotype should be viewed as a minimum estimate.

Variant interpretation continues to be a challenge in clinical genetics.\(^46-48\) Recent studies on HCM have called for more stringent interpretation especially in research studies.\(^15,49,50\) We interpreted variants conservatively using currently accepted American College of Medical Genetics and Genomics guidelines.\(^27\) A subset of VUS identified in this study may be upgraded to likely pathogenic or pathogenic variants with further investigation with family-based segregation studies, which were not performed in this proband only study.

New approaches to better predict variant effects are needed to increase their clinical utility and are particularly important in the context of autosomal dominant diseases such as cardiomyopathy. Our results indicate that additional genetic causes remain to be discovered in pediatric cardiomyopathy.

**CONCLUSIONS**

Our study highlights that a definitive molecular genetic diagnosis can be made in a substantial proportion of
children for whom the cause and heritable nature of their cardiomyopathy have been previously unknown. Overall, our results support use of genetic testing in cases of both familial and idiopathic cardiomyopathy. The diagnostic yields of genetic testing we found can serve as minimum yields for future studies. Additional genetic causes of pediatric cardiomyopathy remain to be discovered. The wide variation in genetic testing practices should be addressed, and improvements can be made in comprehensive cardiac screening and predictive genetic testing in first-degree relatives. Barriers to genetic testing for pediatric cardiomyopathy should be identified and removed to make testing more widely available.

ARTICLE INFORMATION
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Affiliations
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Disclosures
Dr Steven E. Lipshultz is a consultant for Tenaya Therapeutics, Bayer, and on an advisory board for Myokardia.

Supplementary Material
Appendix S1
Tables S1–S4
Figure S1

REFERENCES


Supplemental Material
Appendix

The Pediatric Cardiomyopathy Registry Study Group

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### Table S1. Echocardiography criteria for study eligibility.

**Measurements:** (At least 2 must be present at the time of CM diagnosis for the patient to be eligible, unless otherwise noted)

1) Left ventricular fractional shortening or ejection fraction >2 s.d. below the normal mean for age. Fractional shortening is acceptable in patients with normal ventricular configuration and no regional wall motion abnormalities. Abnormal ejection fraction by echocardiography, radionuclide or contrast angiography, or MRI are acceptable alternatives, but age-appropriate norms for the individual laboratory must be employed.

2) Left ventricular posterior wall or septal thickness at end-diastole >3 s.d. above the normal mean for body-surface area. (This criterion applies to HCM only; Meeting this single criterion qualifies the patient as eligible)

3) Left ventricular end-diastolic posterior wall thickness-to-end-diastolic dimension ratio <0.12.

4) Left ventricular end-diastolic dimension or volume > 2 s.d. above the normal mean for body-surface area. Dimension data are acceptable under the conditions outlined in Measurement Criteria 1 for fractional shortening, and volume data may be derived from the imaging methods as in Measurement Criterion 1.

**Patterns:** (At least 1 must be met for the patient to be eligible if Measurement criteria are not met at the time of CM diagnosis)

1) Localized ventricular hypertrophy, such as septal thickness >1.5 x left ventricular posterior wall thickness with at least normal left ventricular posterior wall thickness, with or without dynamic outflow obstruction.

2) Restrictive cardiomyopathy: one or both atria enlarged relative to ventricles of normal or small size with evidence of impaired diastolic filling and in the absence of significant valvular heart disease.

3) Contracted form of endocardial fibroelastosis, similar to restrictive cardiomyopathy plus echo-dense endocardium.

4) Ventricular dysplasia/Uhl’s anomaly: very thin right ventricle with dilated right atrium usually better assessed by MRI than echo.

5) Left ventricular myocardial noncompaction: very trabeculated spongiform left ventricular myocardium with multiple interstices.
### Inclusion criteria:

1) Patient is alive

   A. Patients who are status-post heart transplant are eligible if pre-transplant longitudinal data is available.

2) Under age 18 years of age at the time of the diagnosis.

3) Either primary or idiopathic Dilated Cardiomyopathy, Hypertrophic Cardiomyopathy, Restrictive Cardiomyopathy, or Left Ventricular Noncompaction.

4) A diagnosis of cardiomyopathy which, at the time of diagnosis, was confirmed by:
   - Echocardiographic criteria, OR
   - Cardiac MRI

### Exclusion criteria:

Arrhythmogenic right ventricular dysplasia

Neuromuscular disease (defined by the specific conditions listed in Appendix A)

Endocrine disease known to cause heart muscle disease (including infants of diabetic mothers)

History of rheumatic fever
<table>
<thead>
<tr>
<th>Toxic exposures known to cause heart muscle disease (anthracyclines, mediastinal radiation, iron overload, or heavy metal exposure)</th>
</tr>
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<tbody>
<tr>
<td>HIV infection or born to an HIV positive mother</td>
</tr>
<tr>
<td>Kawasaki disease</td>
</tr>
<tr>
<td>Immunologic disease</td>
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<tr>
<td>Invasive cardiothoracic procedures or major surgery during the preceding month, except those specifically related to cardiomyopathy including LVAD, ECMO, and AICD placement</td>
</tr>
<tr>
<td>Uremia, active or chronic</td>
</tr>
<tr>
<td>Abnormal ventricular size or function that can be attributed to intense physical training or chronic anemia</td>
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<tr>
<td>Chronic arrhythmia, unless there are studies documenting inclusion criteria prior to the onset of an arrhythmia (except a patient with a chronic arrhythmia, subsequently ablated, whose cardiomyopathy persists after two months is not to be excluded)</td>
</tr>
<tr>
<td>Malignancy</td>
</tr>
<tr>
<td>Systemic hypertension</td>
</tr>
<tr>
<td><strong>Pulmonary parenchymal or vascular disease (e.g., cystic fibrosis, cor pulmonale, or pulmonary hypertension)</strong></td>
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<tr>
<td><strong>Ischemic coronary vascular disease</strong></td>
</tr>
<tr>
<td><strong>Association with drugs known to hypertrophy (e.g., growth hormone, corticosteroids, cocaine)</strong></td>
</tr>
<tr>
<td><strong>Genetic syndrome or chromosomal abnormality known to be associated with cardiomyopathy</strong></td>
</tr>
</tbody>
</table>
Table S3. Exome sequencing metrics. See Excel file.

Table S4. Variants identified in 37 clinically relevant genes. See Excel file.
Figure S1. Variation across the sites.