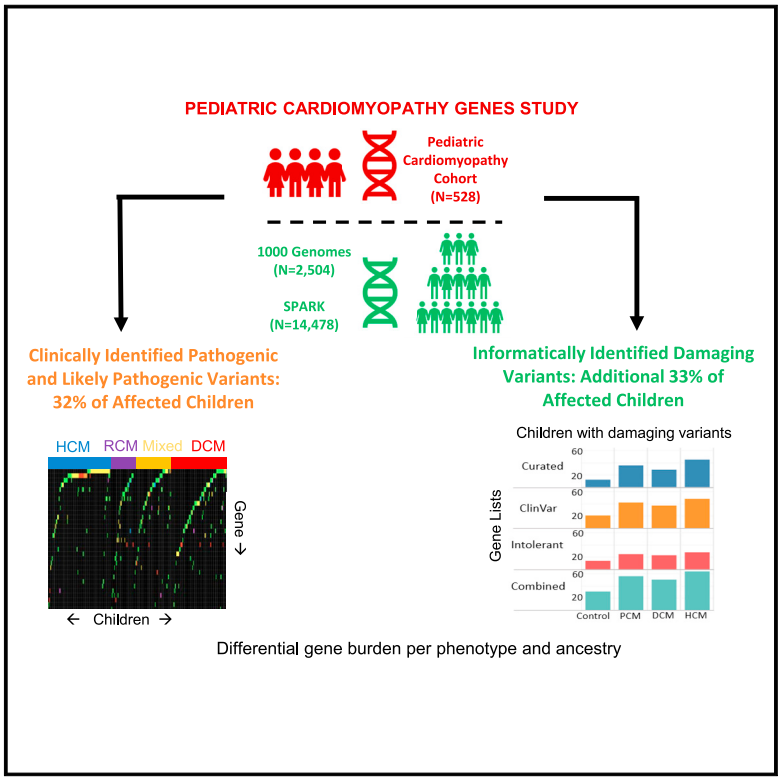
The genetic architecture of pediatric cardiomyopathy

Graphical abstract



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ARTICLE

The genetic architecture of pediatric cardiomyopathy

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Summary

To understand the genetic contribution to primary pediatric cardiomyopathy, we performed exome sequencing in a large cohort of 528 children with cardiomyopathy. Using clinical interpretation guidelines and targeting genes implicated in cardiomyopathy, we identified a genetic cause in 32% of affected individuals. Cardiomyopathy sub-phenotypes differed by ancestry, age at diagnosis, and family history. Infants < 1 year were less likely to have a molecular diagnosis ($p < 0.001$). Using a discovery set of 1,703 candidate genes and informatic tools, we identified rare and damaging variants in 56% of affected individuals. We see an excess burden of damaging variants in affected individuals as compared to two independent control sets, 1000 Genomes Project ($p < 0.001$) and SPARK parental controls ($p < 1 \times 10^{-16}$). Cardiomyopathy variant burden remained enriched when stratified by ancestry, variant type, and sub-phenotype, emphasizing the importance of understanding the contribution of these factors to genetic architecture. Enrichment in this discovery candidate gene set suggests multigenic mechanisms underlie sub-phenotype-specific causes and presentations of cardiomyopathy. These results identify important information about the genetic architecture of pediatric cardiomyopathy and support recommendations for clinical genetic testing in children while illustrating differences in genetic architecture by age, ancestry, and sub-phenotype and providing rationale for larger studies to investigate multigenic contributions.

Introduction

Cardiomyopathy is a rare heart muscle disease that can lead to heart failure and mortality.[1–7] The age of onset of primary cardiomyopathy, defined as disease of the myocardium that does not affect other organs, is highly variable, ranging from infancy to adulthood. Autosomal dominant inheritance of cardiomyopathy in many families provides evidence of a strong genetic component with high penetrance and variable expressivity.[8–10] Gene discovery efforts have implicated variation in sarcomeric genes as a cause of primary cardiomyopathy.

Most gene discovery efforts have been limited to adults. In studies that include both children and adults, a full range of childhood ages is typically not well documented or represented.[11] This is a problem because cardiomyopathy in children is more genetically heterogeneous and can encompass syndromic, metabolic, and neuromuscular causes in addition to primary cardiomyopathies.[6,12–17] While variants in sarcomeric genes are reported in children's cardiomyopathy as well,[18] whether there are pediatric-specific genes is not clear. Indeed, a Finnish study of 66 children with cardiomyopathy referred for transplant evaluation over 20 years identified metabolic, sarcomeric, and syndromic causes in 39% of these sickest of children and identified at least one novel gene associated with disease.[19] Thus, understanding of the genetic causes of primary and idiopathic cardiomyopathy presenting in childhood is still extremely limited and based on studies typically with less than 150 participants. The lack of larger pediatric studies may explain why there is marked practice variation[20–26] despite

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guidelines that recommend genetic testing in children with cardiomyopathy.[12]

The expected genetic heterogeneity and the number of private (or infrequent) variants in pediatric cardiomyopathy present an additional challenge to defining genetic architecture. To be clinically actionable, variants must have multiple tiers of evidence including bioinformatic prediction, clinical phenotyping, familial segregation studies, and functional studies,[27] which makes confirming new disease-causing variants difficult. Furthermore, both nonsynonymous (missense) variants and loss-of-function (LoF) variants may cause the disease, depending on the specific gene, which complicates disease-specific bioinformatic predictions.[8,10,11,28]

When multiple variants (oligogenic inheritance) rather than a single variant act to modify disease risk, these variants may not reach the threshold of clinical actionability due to low penetrance. Thus, to gain understanding of the genetic etiology of pediatric cardiomyopathy, consideration of a broader list of variants beyond those meeting clinical actionability criteria will be required as well as non-Mendelian inheritance models such as gene burden. As pediatric cases are rarer than adult cardiomyopathy cases (1 in 100,000 compared to 1 in 500), targeted discovery approaches will be essential as the number of pediatric cases will be orders of magnitude lower than in adult studies. Systems biologic approaches have been shown to effectively leverage current biological knowledge to inform which genes have the highest potential of contributing to cardiomyopathy and to reduce multiple testing burden.

Given the relatively limited data on the genetics of cardiomyopathy in children, the purpose of this paper was to investigate the genetic architecture of pediatric-onset cardiomyopathy. To address this question, we analyzed a large cohort of children with cardiomyopathy in North America. We determine likely genetic causes, identifying the yield of testing by cardiomyopathy sub-phenotype, age of diagnosis, and ancestry. Second, we provide an exome-based assessment of the genetic architecture of pediatric cardiomyopathy and identify an over-representation of bioinformatically predicted damaging variant burden, some of which is ancestry dependent. These findings facilitate deeper insight into the genetic architecture of pediatric cardiomyopathy.

Subjects and methods

Cohort composition and exome sequencing

Participants with pediatric cardiomyopathy were recruited from 14 sites in the United States and Canada. The procedures followed were in accordance with the ethical standards and the responsible conduct on human and experimentation and was approved by the institutional review board (institutional and national). Proper informed consent was obtained. The research methods, including eligibility criteria, sample handling, and exome sequencing procedures, are described elsewhere.[26] Briefly, individuals with familial or idiopathic hypertrophic cardiomyopathy (HCM [MIM: 192600]), dilated cardiomyopathy (DCM [MIM: 115200]), restrictive cardiomyopathy (RCM [MIM: 115210]), or left ventricular noncompaction (LVNC [MIM: 604169]) were eligible if the diagnosis was made before age 18. Individuals with LVNC sub-phenotype (n = 17) or LVNC with HCM, DCM, and/or RCM in combination were given the designation of "LVNC/mixed." Individuals with more than one sub-phenotype without LVNC in combination were given the designation of "non-LVNC mixed" sub-phenotype. Exome sequencing was performed at Cincinnati Children's Hospital Medical Center with Nimblegen sequence capture (SeqCap EZ Human Exome 2.0) and an Illumina HiSeq2500. The mean sequence coverage over all samples was 79× (range: 31 to 155). Alignment was performed as described previously.[26]

Ancestry estimation

To estimate ancestry, variants with a minor allele frequency (MAF) greater than 10% were identified in the dataset. These variants were linkage disequilibrium (LD) pairwise pruned with the PLINK procedure (window size, 50; step, 5; r^2 threshold, 0.5). From the LD-pruned variants, 5,000 variants were randomly selected. Because principal-component analysis (PCA) can be performed only on complete data, SNPs not called in all pediatric cardiomyopathy (PCM) cohort samples were excluded (the final sample was 3,027 variants). We performed PCA on these SNPs in the 1000 Genomes dataset to establish super population clusters. The distance from each population centroid with three principal components was calculated. Ancestry for PCM participants was based on being localized within 3 standard deviations of the population centroid.

Clinical variant interpretation of curated genes

At study initiation and participant enrollment from 2013–2016, 37 genes were curated from the literature and from available clinical genetic testing panels as genes in which pathogenic variation is potentially causative in infants and children with idiopathic or familial cardiomyopathy (Table S1). Two independent bioinformatic groups (CCHMC and CUMC) identified rare variants (MAF < 0.005) for further classification with the PCM exome files. Variants (N = 549) in this 37 gene curated gene list were interpreted as per American College of Medical Genetics and Genomics (ACMG) clinical-variant interpretation guidelines (Table S1).[27] Given the large number of variants in *TTN* (MIM: 188840) and the strong evidence for truncating variants causing DCM, variant interpretation was limited to nonsense and frameshift variants within the A-band region of the protein.[29,30] Variant interpretations by the two bioinformatic groups were 98% concordant with adjudication between the two groups performed for the remaining seven variants to arrive at consensus interpretation. The curated gene set and variant interpretations were frozen January 2019 and used for subsequent analyses. The variant results and interpretation criteria are provided in Table S1. Variant reinterpretation was performed October 2021 for likely pathogenic (LP) and pathogenic (P) variants as noted in Table S1.

Compiling cardiac discovery gene list and sub-lists

Curated gene

The curated gene set included the following 37 genes: *ABCC9* (MIM: 601439), *ACTC1* (MIM: 102540), *ACTN2* (MIM: 102573), *ANKRD1* (MIM: 609599), *BAG3* (MIM: 603883), *CAV3* (MIM: 601253), *CRYAB* (MIM: 123590), *CSRP3* (MIM: 600824), *DES* (MIM: 125660), *EMD* (MIM: 300384), *LAMP2* (MIM 309060), *LDB3* (MIM: 605906), *LMNA* (MIM: 150330), *MYBPC3* (MIM:

600958), *MYH6* (MIM: 160710), *MYH7* (MIM: 160760), *MYL2* (MIM: 160781), *MYL3* (MIM: 160790), *MYPN* (MIM: 608517), *NEBL* (MIM: 605491), *NEXN* (MIM: 613121), *PLN* (MIM: 172405), *PRKAG2* (MIM: 602743), *RBM20* (MIM: 613171), *SCN5A* (MIM: 600163), *SCO2* (MIM: 604272), *SGCD* (MIM: 601411), *SURF1* (MIM: 185620), *TAZ* (MIM: 300394), *TCAP* (MIM: 604488), *TNNC1* (MIM: 191040), *TNNI3* (MIM: 191044), *TNNT2* (MIM: 191045), *TPM1* (MIM: 191010), *TTR* (MIM: 176300), *VCL* (MIM: 193065), *TTN* (MIM: 188840). We next compiled multiple gene lists of cardiac discovery genes by using multiple primary sources, including the Online Mendelian Inheritance in Man (OMIM) compendium, ClinVar data, the Gene Ontology (GO) initiative, UniProt data, functional domains, and phenotype associations through the ToppGene Suite (Figure S1).[31,32] We focused on finding and aggregating additional genes either known or potentially associated with cardiomyopathy, heart development, and cardiac muscle structure by using available human phenotype, mouse phenotype, and co-expression data. The provenance of the gene lists is detailed in Table S2. This search identified our broadest list of 1,703 potential cardiac discovery genes. We also compiled several smaller lists within the cardiac discovery gene set. Table S3 provides each gene list.

ClinVar gene set

The ClinVar gene list consisted of 70 genes associated with cardiomyopathy having a P or LP variant (ClinVar Version August 2020): *ABCC9*, *ACTC1*, *ACTN2*, *ALPK3* (MIM: 617608), *BAG3*, *BRAF* (MIM: 164757), *CRYAB*, *CSRP3*, *DES*, *DMD* (MIM: 300377), *DPM3* (MIM: 605951), *DSG2* (MIM: 125671), *DSP* (MIM: 125647), *DTNA* (MIM: 601239), *EYA4* (MIM: 603550), *FKTN* (MIM: 607440), *FLNC* (MIM: 102565), *GATAD1* (MIM: 614518), *GLA* (MIM: 300644), *HAND2* (MIM: 602407), *JPH2* (MIM: 605267), *LAMA4* (MIM: 600133), *LAMP2*, *LDB3*, *LIMS2* (MIM: 607908), *LMNA*, *MIPEP* (MIM: 602241), *MYBPC3*, *MYH6*, *MYH7*, *MYL2*, *MYL3*, *MYLK2* (MIM: 606566), *MYO6* (MIM: 600970), *MYOZ2* (MIM: 605602), *MYPN*, *MYZAP* (MIM: 614071), *NCF1* (MIM: 608512), *NDUFB11* (MIM: 300403), *NEXN*, *NKX2-5* (MIM: 600584), *PKP2* (MIM: 602861), *PLN*, *PMPCA* (MIM: 613036), *PPC3* (MIM: 609853), *PRDM16* (MIM: 605557), *PRKAG2*, *PSEN1* (MIM: 104311), *PTPN11* (MIM: 176876), *RAF1* (MIM: 164760), *RBM20*, *RYR2* (MIM: 180902), *SCN1B* (MIM: 600235), *SCN5A*, *SCO2*, *SDHA* (MIM: 600857), *SDHD* (MIM: 602690), *SGCD*, *TAZ*, *TCAP*, *TMEM43* (MIM: 612048), *TNNC1*, *TNNI3*, *TNNI3K* (MIM: 613932), *TNNT2*, *TPM1*, *TSFM* (MIM: 604723), *TTN*, *TTR*, *VCL*.

LoF-intolerant gene set

The LoF-intolerant gene set is the genes within the cardiac discovery gene set that are highly intolerant to a LoF variant. The Exome Aggregation Consortium (ExAC) uses the observed and expected variant counts to determine the probability that a given gene is highly intolerant to haploinsufficiency.[33,34] The probability of LoF intolerance (pLI) ranges from 0 to 1, where 1 indicates complete intolerance. To identify genes that are highly intolerant to LoF variants, we used a pLI [ExAC] score of 0.9 or greater. There were 457 genes found in cardiac discovery with a pLi > 0.9, of which 18 genes were found to have a damaging LoF variant per CADD[35] > 20.

Missense-intolerant gene set

The missense-intolerant genes are the genes within the cardiac discovery gene set that are highly intolerant to a damaging missense variant. Given the high frequency of missense variants, we developed a damaging missense ratio (see methods below) to identify genes specifically intolerant to damaging missense variants; unlike MisZ [ExAC], which can be used to identify genes intolerant to any missense variant, we used the top 20% of the ranked genes in the cardiac discovery gene set to create our missense-intolerant gene list. There were 337 genes found in cardiac discovery with a missense-intolerant score in the top 20 percentile, of which 89 genes were found to have a damaging missense variant per Meta-SVM.

Damaging missense ratio

To evaluate the tolerance of pathogenic-like variants within a gene, we compiled the number of synonymous variants and the number of damaging missense variants (as per Meta-SVM) seen in our 1,703 genes of interest across the participants in the 1000 Genomes data. We ranked the genes by taking the ratio of non-synonymous damaging variation to synonymous variation and selected 20% of the genes as those most intolerant to damaging non-synonymous variation. The combined analysis includes all damaging variants found across LoF-intolerant, missense-intolerant, curated, and ClinVar gene lists (Table S3, Figure S1).

Control cohorts

1000 Genomes

1000 Genomes Phase 3 individuals (n = 2,504) were used as control individuals.[36] We also analyzed the cohort by super population ancestry per 1000 Genomes: 503 European (EUR), 347 admixed American (AMR), and 661 African (AFR) individuals. In PCA (Figure S2), we observed that individuals in the PCM cohort overlap only a portion of the African population in 1000 Genomes. Therefore, we limited our analysis to the African ancestry of Southwest USA (ASW; n = 61) within the 661 African (AFR) population in 1000 Genomes to better match the genetic background of our African American participants.

Random genes in the Pediatric Cardiomyopathy cohort

As an additional control, we examined the damaging variant burden of 1,703 cardiac discovery genes compared to the average damaging variant burden of 1,703 random genes over 1,000 iterations. We performed the same analysis for all gene lists. We also compared burden distributions of damaging variants in individuals for selected and random genes for all gene lists.

Simons Foundation Powering Autism Research for Knowledge (SPARK)

Control individuals (n = 14,478) from unrelated parents in SPARK were used. The case and control samples were called with GATK[37] and jointly with GLnexus. We used the same principal components calculated from the 1000 Genomes dataset to estimate the ancestry for the SPARK control individuals. Control individuals were matched to affected individuals with the smallest Euclidian distance in the PCA space, resulting in 9,150 European (EUR), 1,920 admixed American (AMR), and 425 African American (AFR) individuals.

Study design of exploratory analysis of damaging variants

The clinical variant evaluation guided our approach to use MAF $<$ 0.001 (overall and population ancestries) for affected individuals and control individuals in the informatics pipeline. To filter variants that occurred at a higher rate than would be consistent with a variant causing cardiomyopathy, we evaluated the ancestry-specific MAF reported in gnomAD.[38] To determine the appropriate minor allele threshold for consideration, we evaluated the MAF of the P/LP variants from our clinical variant interpretation and found that only three variants exceeded a minor allele frequency equaling 0.001 based on the gnomAD ancestry-specific

MAF. Thus, we excluded variants that exceeded a MAF of 0.001 in any of the ancestry-specific gnomAD MAF. We excluded variants that did not pass gnomAD filters. We also excluded lower quality variants observed in gnomAD in less than 80% of the exomes i.e., variants with allele number (AN) less than 80% of total allele count. We eliminated variants located in regions with low or no coverage for the Nimblegen Exome Enrichment platform used for the PCM cohort. We also excluded variants that failed GATK filtering for the complete cohort (n = 528 individuals) but had passed GATK filtering during initial clinical evaluation (n = 152 individuals).[26] Since 1000 Genomes Project data were based on multiple platforms and about 20% of their genotypes are imputed, traditional exome workflows may miss many variants reported in these data. Therefore, we applied additional cohort-specific filters to remove any bias caused by different sequencing platforms. For the PCM exomes, we excluded variants that did not pass our GATK variant calling and applied a filtering allele frequency of 12 alternate alleles or less. We only considered genotypes with a GQ score of 20 or greater. For the 1000 Genomes data, we applied a filtering allele frequency of four alternate alleles or less for EUR ancestry, two alternate alleles or less for AMR ancestry, and two alternate alleles or less for AFR ancestry. Given that the sample size of gnomAD is about 50 times the size of the 1000 Genomes Project, variants captured by traditional exome sequencing were most likely detected. Therefore, if a variant had a reported 1000 Genomes frequency greater than 100-fold that of gnomAD, it was excluded as artifact. We performed quality control metrics to assess exome-wide missense, synonymous, and LoF variants in autosomes in affected individuals and control individuals after applying cohort-agnostic and cohort-specific filters.

Damaging missense variants

For missense variants, we considered rare (MAF $\leq$ 0.001) variants called "deleterious" by Meta-SVM, with a reliability of 0.5 or higher that passed the above-mentioned quality and control filters. We selected Meta-SVM after analyzing several tools and their accuracy at predicting P and LP variants in ClinVar[39] associated with cardiomyopathy.

Damaging LoF variants

For LoF variants, we considered rare (MAF $\leq$ 0.001) variants with a CADD Phred score[40] of at least 20 that passed the above-mentioned quality and control filters. The other pathogenicity prediction tools we considered could not make appropriate calls for LoF variants.

Statistical methods used

Dichotomous outcomes are reported as frequencies and continuous outcomes reported as medians and interquartile ranges (IQRs) unless otherwise specified. Descriptive statistics were calculated overall and by type of cardiomyopathy: DCM, HCM, RCM, LVNC/mixed (including LVNC only or LVNC combined with other cardiomyopathy types), and non-LVNC mixed cardiomyopathy sub-phenotype. To compare the rates of positive clinical genetic findings, we used contingency tables with goodness of fit tests. To estimate pairwise effects, we estimated odds ratios as well.

Our control populations in 1000 Genomes and our PCM cohort were processed differently. Therefore, we used a strict internal quality control to ensure our findings were not biased by these differences. In addition, we compared the overall number of synonymous and missense variants between the cohorts. To test whether individuals with cardiomyopathy in the PCM cohort had a higher burden of damaging variants (as predicted with bioinformatics) in our gene lists (curated, ClinVar, intolerant, combined, and full cardiac discovery) compared to the control population in 1000 Genomes, we used Wilcoxon rank-sum tests. To compare the cardiomyopathy sub-phenotypes against 1000 Genomes control, we also used Wilcoxon rank-sum tests for continuous outcomes. All statistical tests used were 2-tailed. To evaluate burden by ancestry within the PCM cohort, we used logistic regression and tested whether ancestry (restricted to EUR and AFR participants) was associated with the presence of one or more damaging variants. As cardiomyopathy type exhibited substantial differences in the analysis of our clinical variants, we also evaluated models with cardiomyopathy type as a covariate in the logistic model.

We also used the random genes in PCM as a control set. For this control, we compared the variant burden observed when selecting an equal number of genes randomly 1,000 times. We used pairwise Wilcoxon rank-sum tests for each replicate and calculated the median p value across the replicates. This was done separately by ancestry for each gene list. As most of the gene lists had overall low burden, we reported the median, as well as the 75th and 90th centiles rather than the IQR.

For the case-control burden analyses (both using control cohorts as well as the randomly selected genes), we performed 12 and 15 tests for control cohorts and randomly selected genes, respectively, when comparing the overall PCM cohort. Thus, the Bonferroni-corrected threshold for cohort analyses is $p \leq 0.0042$ and for random gene list is $p \leq 0.0033$.

Results

Sex, ancestry, and age differences in pediatric cardiomyopathy sub-phenotypes

The pediatric cardiomyopathy (PCM) cohort comprises 528 participants with cardiomyopathy who underwent exome sequencing (Table 1), 54% of whom are males. The cardiomyopathy sub-phenotypes differ with regard to sex, and HCM occurs more frequently in males (69%). Using data from the 1000 Genomes Project,[36] PCA assigned genetic ancestry to all participants (Figure S2). Self-reported race was highly concordant (99%) with continental ancestry. Ancestry for distinct cardiomyopathy sub-phenotypes differed markedly (Table 1), and European ancestry was more common in HCM and RCM. The age of diagnosis also varied significantly by sub-phenotype, and DCM and LVNC/mixed type occurred commonly in very young children (median age of diagnosis, 1.3 and 1.7 years, respectively) compared to a median age of diagnosis of 11.1 years in HCM. Participants with HCM had the highest frequency of a family history of cardiomyopathy, and 57% had at least one affected family member (Table 1).

Diagnostic genetic findings

At study initiation, 37 genes were identified for potential return of results to participants. After exome sequencing, 549 distinct variants (1,005 total instances) were evaluated from these 37 genes for pathogenicity using ACMG guidelines (Table S1).[27] After review, variants were classified as 127 unique P or LP, 308 unique variants of uncertain significance (VUSs) (Table S1; 176 total P/LP and 356 total VUS

Table 1. Demographic information of 528 children with cardiomyopathy

Trait	All, 528 (100%)	DCM, 279 (52.8%)	HCM, 160 (30.3%)	RCM, 30 (5.7%)	LVNC/ mixed, 53 (10.0%)	Mixed not LVNC, 6 (1.1%)	p value
Male, %	53.6	46.6	69.4	50.0	49.1	16.7	<0.001
Ancestry, %[a]							
European	58.0	50.2	71.3	80.0	45.3	66.7	<0.001
African	16.1	17.6	11.3	3.3	32.1	32.1	
Asian	1.7	2.9	0.6	0	0	0	
Admixed American	24.2	29.4	16.9	16.7	22.6	33.3	
Age at diagnosis, median (IQR), years	3.2 (0.4–12.8)	1.3 (0.3–9.4)	11.1 (3.2–14.0)	8.3 (1.7–13.2)	1.8 (0.2–12.5)	0.80 (0.1–12.3)	<0.001
Family history of CM	36.0	24.2	57.2	28.6	35.9	50.0	<0.001

DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; RCM, restrictive cardiomyopathy; LVNC, left ventricular non-compaction; CM, cardiomyopathy; IQR, interquartile range.
[a]Ancestry was defined via principal-component analysis with 1000 Genomes superpopulations.

instances) and 114 unique benign/likely benign (B/LB, 473 total B/LB instances). Missense variants (91.5%) were more common than LoF variants (5.4%) and inframe variants (3.1%).

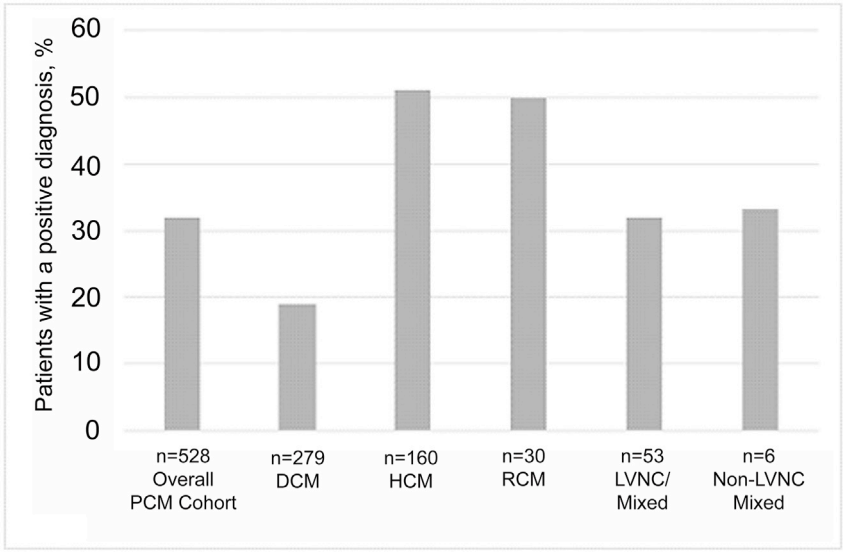
Overall, 32% of the PCM cohort had a positive result, i.e., P/LP variant (Figure 1), and another 35% had a VUS. Only 1.3% of participants carried multiple P/LP variants in different genes. Of the 303 participants (57%) with no previous clinical genetic testing, 75 (25%) of these had P/LP variants in one of the 37 known cardiomyopathy genes. Diagnostic yield was lower for DCM (19%) than for RCM (50%) or HCM (51.3%; Figure 1; $p < 0.001$). Diagnostic yield was also higher for European ancestry than admixed American ancestry (OR, 2.0; 95% CI, 1.3–3.3; $p = 0.0032$) and showed a similar trend with higher yield in European compared with African American ancestry (OR, 1.6; 95% CI, 0.94–2.7; $p = 0.08$) (Figure 1B). These findings should be interpreted in the context of a smaller cohort size of African American ancestry and the fact that distinct cardiomyopathy subphenotypes differed by ancestry.

Participants aged 0 to 5 years had the lowest frequency of P/LP genetic findings (Figure S3). We then compared the frequency of molecular diagnoses between participants diagnosed with cardiomyopathy as infants (12 months or younger) and older children. Infants had lower rates of positive genetic results overall when treating cardiomyopathy type as a strata variable ($p < 0.001$). When stratifying by cardiomyopathy subtypes, infants tended to have lower diagnostic yield, but none of the individual comparisons was statistically significant; HCM ($p = 0.053$), DCM ($p = 0.07$), and LVNC/mixed ($p = 0.06$; Figure 1C).

From the 37 cardiomyopathy-associated genes in the curated gene list, we identified P/LP variants in 22 genes (Tables S1 and S4). Variants in the sarcomeric genes *MYH7* and *MYBPC3* were the most common causes, with 56 and 35 variants, respectively (Table S4). Notably, no African American ancestry participants had *MYBPC3* variants compared to 8.8% ($n = 27/306$) in the European or 3.9% ($n = 5/128$) in the admixed American cohorts. The African American cohort, albeit small, had a significantly lower rate of *MYPBC3* variants compared to European Americans ($p = 0.0014$). Restricting the analysis to HCM, there were 23 of 114 European ancestry HCM children with *MYBPC3* P/LP variants as compared to 0 of 18 African American ancestry ($p = 0.042$). Eight African American ancestry participants with DCM or LVNC/mixed carried the p.Val142Ile TTR (rs76992529 [GenBank: NM_000371.3; c.424G>A]) pathogenic variant (gnomAD[38] AFR MAF = 0.0162) known to cause cardiac amyloidosis in older adults. This variant was not considered causal in these participants given their age and cardiomyopathy sub-phenotype. Three of these individuals had other P/LP variants that explained their disease. In HCM, phenocopy genes, non-sarcomeric genes whose variants cause ventricular hypertrophy, have been identified in ~3% of HCM samples with diagnostic findings.[11] We identified one pathogenic variant in *LAMP2*, the cause of Danon disease and two pathogenic variants in *PRKAG2* encoding protein AMP kinase gamma-2, resulting in glycogen storage disease of the heart. In DCM samples, pathogenic variants were identified in *BAG3*, *CRYAB*, and *DES*, all of which are associated with myofibrillar myopathy and may, in some instances, have skeletal muscle findings in addition to DCM (Table S4).

Gene-specific rates of P/LP variants were similar to rates in adults for many well-established cardiomyopathy genes, but we also found results that differed from adults for RCM and DCM (Table 2). In HCM, P/LP variants in sarcomeric genes predominated in our pediatric participants as they do in adults. Specifically, we identified *MYH7* P/LP variants in 23.1% of our HCM participants, whereas yields previously reported in adults are 16% to 19%. The overall yield in HCM was 51.3% in our participants and 32% in a large HCM cohort from an academic genetic

A



B

Ancestry	PCM	PCM Phenotype				
	PCM (528)	DCM (279)	HCM (160)	RCM (30)	LVNC/ Mixed (53)	Non-LVNC Mixed (6)
EUR	37.3	20.0	53.5	54.2	45.8	25.0
AMR	22.7	15.9	48.1	20.0	8.3	50.0
AFR	27.1	20.4	38.9	100.0	29.4	0.0

C

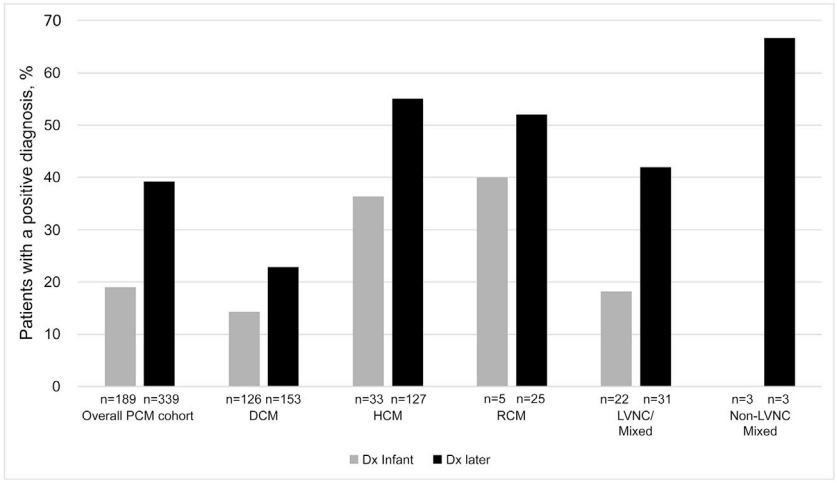


Figure 1. Diagnostic yield of exome sequencing

(A–C) Exome data were filtered for 37 known cardiomyopathy-associated genes, and variants were classified per clinical guidelines to identify pathogenic or likely pathogenic variants, the presence of which was considered a positive result. (A) Overall diagnostic yield. (B) Diagnostic yield by ancestry. (C) Diagnostic yield in infants less than 1 year old (gray bars) and older children (black bars), by subtype of cardiomyopathy. The number of participants in each age group is shown below each bar. DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LVNC, left ventricular noncompaction; PCM, pediatric cardiomyopathy; RCM, restrictive cardiomyopathy.

testing laboratory, including a yield of 28% in their 462 individuals with HCM age 16 or less.[9] Because RCM is so rare, gene-specific frequencies in adults are not available. In our cohort, *TNNI3* and *MYH7* had the most P/LP variants among individuals with RCM, and variants were also identified in *TNNT2*, *DES*, and *MYL2*. In children with DCM, no one gene accounted for more than 4% of positive findings. In adults, DCM is also a genetically heterogeneous disorder, and gene-specific diagnostic rates are low except for variants in *TTN* that account for 10%–20% of adult DCM (Table 2). Rates of P/LP variants in *MYH7*, *MYBPC3*, *TNNT2*, and *RBM20* were similar to adults. However, we found lower rates in *LMNA* and *TTN*. P/LP variants were seen in *LMNA* at a frequency of 1.4% in our cohort versus 6% in adult cohorts and *TTN* was seen at 3.2% in our cohort versus 10% to 20% in adult cohorts. The average age of the nine children with *TTN* P/LP variants in our cohort was 12.4 years (range 1.2–15.1) and two children were African American and seven were non-Hispanic White.

The number of P/LP and VUS variants per participant for the curated gene list (37 genes) showed that P/LP variants are more commonly missense (yellow) than LoF (red) when clustered across sub-phenotypes and ancestries (Figure S4). VUSs are also typically missense variants (green) and are less often seen in individuals with HCM. HCM caused by *MYH7* or *MYBPC3* variants can be segregated into one subset with multiple additional VUS findings and a second subset with no additional variants. Overall, when considering both P/LP and VUS variants, DCM, LVNC/mixed, and mixed phenotypes show high rare variant burden across these 37 genes implicated in cardiomyopathy.

Development of a cardiac discovery gene list and informatic analysis of damaging variants

To expand to additional known and candidate cardiomyopathy genes, we identified 70 cardiomyopathy-associated genes in ClinVar,[39] 32 of which were present in our curated gene list (Table S3 and Figure S1) and combined them with

Table 2. Differences in the genetic causes of cardiomyopathy between children in the pediatric cardiomyopathy cohort and adults

Gene	Adults with HCM, range, %[a]	Children with HCM, % (95 CI)	Children with RCM, % (95 CI)	Adults with DCM, %[b]	Children with DCM, % (95 CI)
MYH7	16–19.2	23.1 (17.3–30.2)	10.0 (3.5–25.6)	4	2.9 (1.5–5.6)
MYBPC3	16–26	17.5 (12.4–24.1)	0	2–4	0.4 (0.06–2.0)
TNNI3	1.6–3	1.3 (0.3–4.4)	30 (16.7–47.9)	1	0.4 (0.06–2.0)
TNNT2	1.3–3.4	1.3 (0.3–4.4)	3.3 (0.5–16.7)	3	2.9 (1.5–5.6)
DES	unknown	0.6 (0.1–3.5)	3.3 (0.5–16.7)	<1	0.4 (0.06–2.0)
TPM1	1.1	3.1 (1.3–7.1)	0	<1–2	1.1 (0.4–3.1)
RBM20	N/A	0	0	2	1.8 (0.8–4.1)
LMNA	N/A	0	0	6	1.4 (0.6–3.6)
MYL2	unknown	0.6 (0.1–3.5)	3.3 (0.6–16.7)	unknown	0.7 (0.2–2.6)
TTN	N/A	0	0	10–20	3.2 (1.7–6.0)

HCM, hypertrophic cardiomyopathy; RCM restrictive cardiomyopathy; DCM, dilated cardiomyopathy; N/A, not applicable.
[a]Derived from Ingles et al.[41] and Alfares et al.[11]
[b]Derived from GeneReviews (see web resources).

genes curated from available ontologies. The provenance of the 1,703 cardiac discovery genes is shown in Table S2 and each gene set is provided in Table S3. To reduce noise, we identified genes intolerant to variation by using the pLI metric to select and rank LoF-intolerant genes (N = 459 genes)[33] and derived an analogous metric for missense variants by assessing the ratio of damaging missense variant burden to synonymous variant burden in the 1000 Genomes cohort. We used this metric to rank genes intolerant to damaging missense variation (N = 339 genes). The combined analysis includes all damaging variants found across curated, ClinVar, LoF-intolerant, and missense-intolerant gene lists (Table S5). For variant classification and interpretation, a process that is labor intensive and not easily scalable, we utilized a bioinformatic approach that prioritized damaging variants by combining variant quality attributes with prediction tools to enhance classification (Figure S5). As a proof of principle, we tested for concordance between clinical variant interpretation and our bioinformatic approach using the curated gene list. Of the 549 variants interpreted clinically, 544 also had bioinformatic interpretation. The bioinformatic approach correctly identified 116 out of 124 P/LP variants as damaging and 108 out of 114 benign (B)/likely benign (LB) as tolerated (Figure S5A). Using only the P/LP and B/LB variant calls, the informatic approach has 94% sensitivity and 95% specificity (Figure S5A). The damaging variant burden analysis uses both cohort agnostic and cohort-specific filters (Figure S5B). As an additional control of the bioinformatic variant filtering, we assessed metrics by variant type and by ancestry on an exome-wide basis (Figure S5C), demonstrating that at an exome-wide level there is no variant bias between affected individuals and control individuals.

Assessing damaging variant burden within the ClinVar gene set highlights the skewed distribution seen across sub-phenotypes and ancestries (Figure 2). These findings correspond to gene-phenotype correlations recognized in clinical practice and by our manual interpretation of variants via ACMG guidelines. For example, genes such as *MYH7*, *FLNC*, *TNNI3*, and *TNNT2* have damaging variants in all sub-phenotypes, although their distribution varies and *TNNI3* damaging variants are again noted as prominent in RCM. Other genes, such as *MYBPC3*, *PKP2*, *DSP*, and *RBM20* predominate in a specific sub-phenotype. The results also demonstrate that predicted damaging variants in phenocopy genes can be identified.

Excessive damaging burden identified in the cardiac discovery gene list

We hypothesized that genes involved in cardiomyopathy would collectively have more damaging variants in the PCM cohort than in a control group. To address this question, we investigated the odds of harboring a damaging variant in PCM affected individuals compared to 1000 Genomes participants, as well as in HCM and DCM, the two sub-phenotypes with the largest numbers (Figure 3). The odds ratio for damaging variants in PCM is greater than 1000 Genomes, although several confidence intervals overlap one in the non-European ancestral groups (Figure 3A). Of note, the magnitude of the odds ratio was higher for HCM than DCM, consistent with the clinical findings. We also see higher odds of having a damaging variant in PCM participants compared to a second control dataset, SPARK (Figure 3B).[42] In the SPARK controls, HCM again demonstrates a higher magnitude odds ratio than DCM.

As the comparisons between the PCM cohort and both sets of control individuals yielded higher magnitude odds ratio in individuals of European descent, we then compared the likelihood of having at least one damaging variant among the curated, ClinVar, intolerant, and combined gene lists within the PCM cohort between European

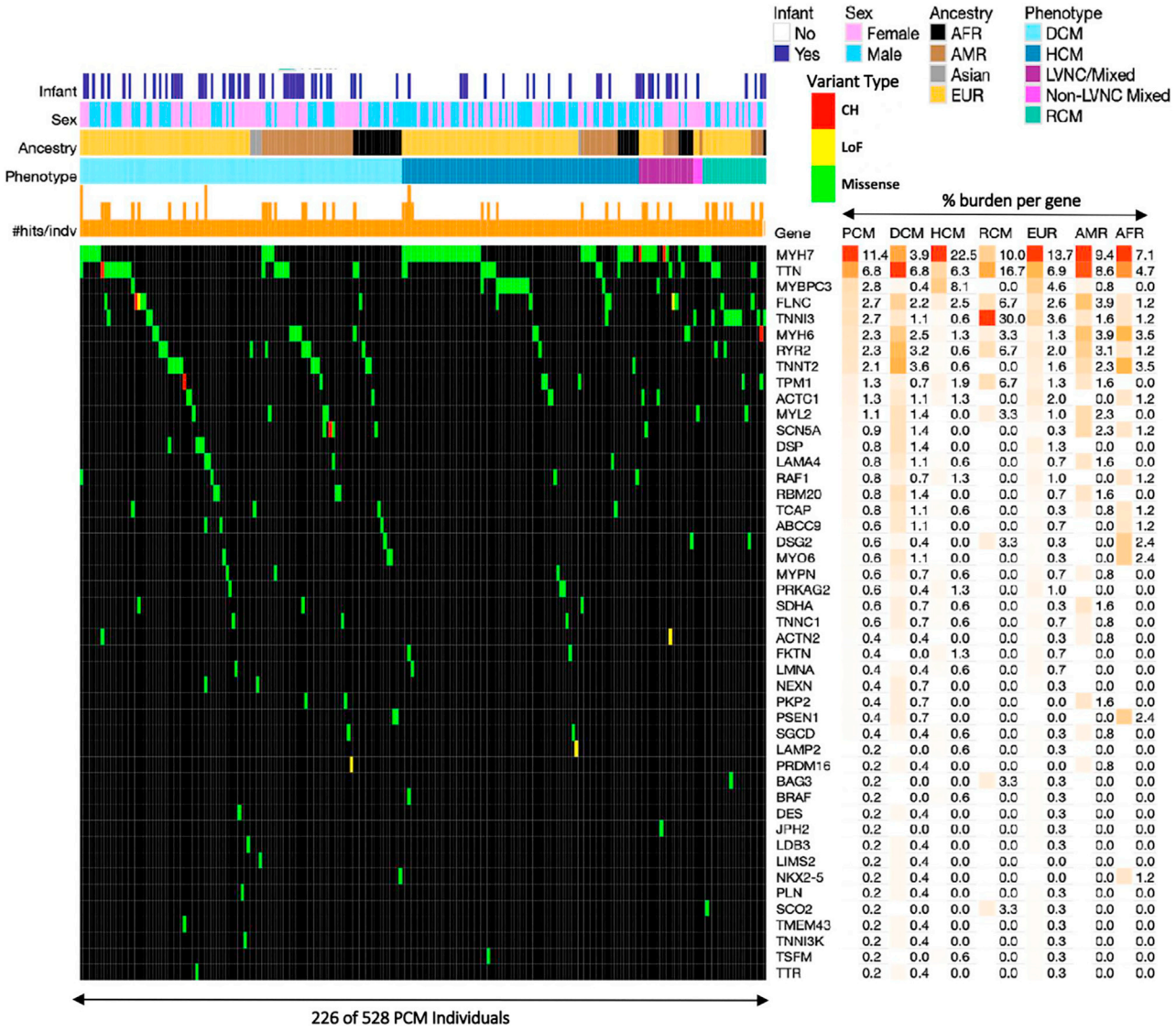


Figure 2. Damaging variants in the ClinVar genes clustered by phenotype, ancestry, and gene

Each row represents an individual participant and each column represents a gene. Variants are color-coded as indicated based by their variant classification. DCM participants are clustered first, followed by those with HCM, LVNC/mixed, non-LVNC mixed, and RCM cases. Participants with damaging variant findings are shown (226/528 individuals). CH, compound heterozygous (*cis*/trans configuration unknown); DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LoF, loss of function; LVNC, left ventricular noncompaction; RCM, restrictive cardiomyopathy.

and African descent individuals (Figure 3C). Individuals of European ancestry were significantly more likely than individuals with African ancestry to have at least one damaging variant in the curated gene list (OR = 2.18, p = 0.0054) and an attenuated effect when using the ClinVar list (OR = 1.63, p = 0.057). Notably, when including type of cardiomyopathy as a covariate, the effect is lessened for the curated gene list (OR = 1.79, p = 0.046) and ClinVar (OR = 1.37, p = 0.23). No ancestry differences were present in the intolerant and combined gene lists.

As the odds ratios are a function of both the rate of the affected individuals and control individuals, we also evaluated the frequencies of harboring at least one damaging variant across the gene sets. Given the similarity in the odds ratios between 1000 Genomes and SPARK, we report only the 1000 Genomes control individuals. Among the curated gene list, this bioinformatic approach identified 643 damaging variants in PCM and 1000 Genomes (Table S5). Of note, there was no overlap of damaging variants between PCM affected individuals and 1000 Genomes control individuals except in *TTN* (Table S5). *TTN* variants were the largest contributor to predicted damaging variants in control individuals (N = 148 unique to 1000 Genomes affected individuals). The rates of harboring a damaging variant varied considerably across the PCM subtypes (Figure 4A). Further, there was marked variability across the ancestries, especially when considering cardiomyopathy subtype (Figure 4B). Among the ClinVar gene list (N = 70 genes), we identified 946 damaging variants in PCM and 1000 Genomes with marked variation in the

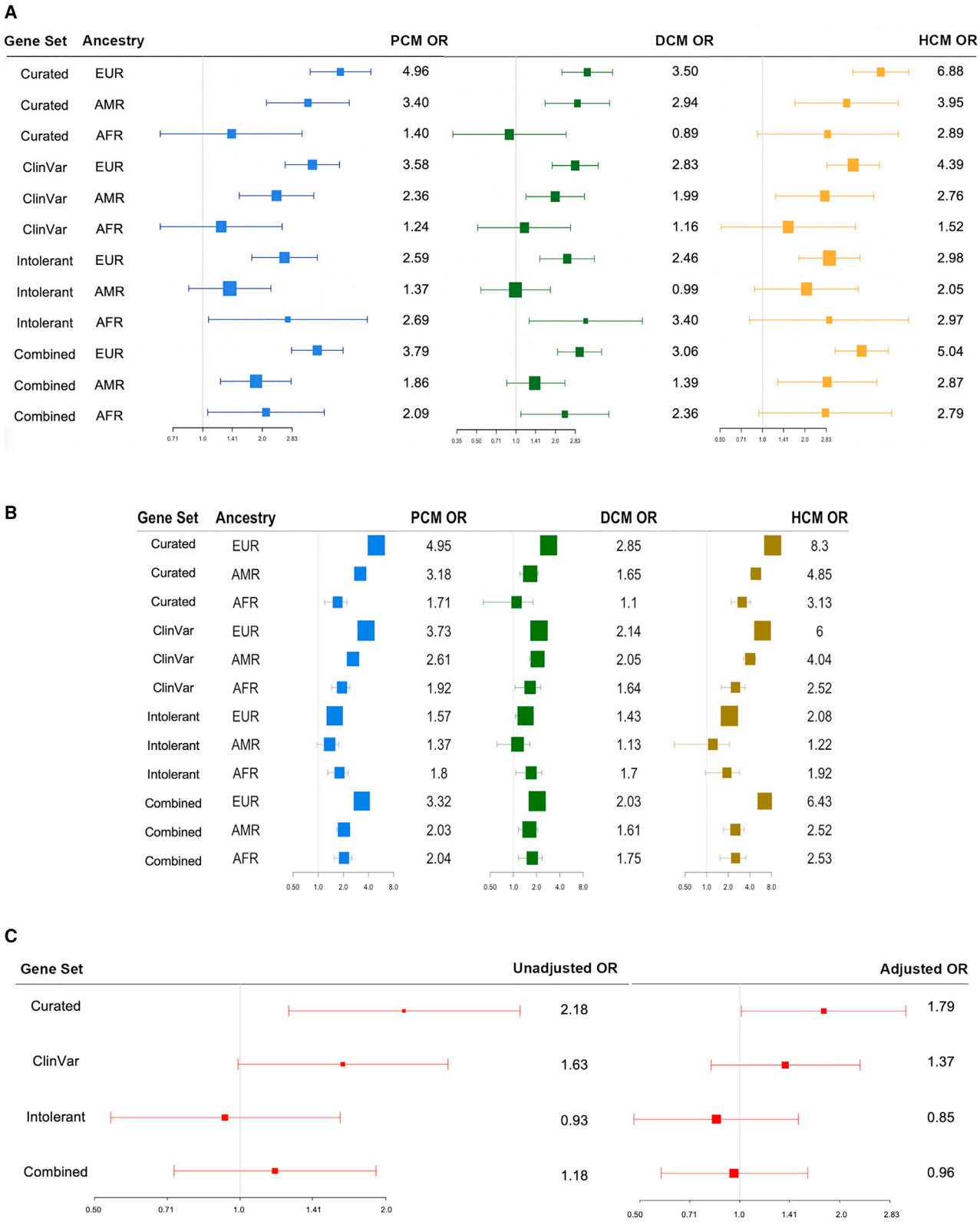
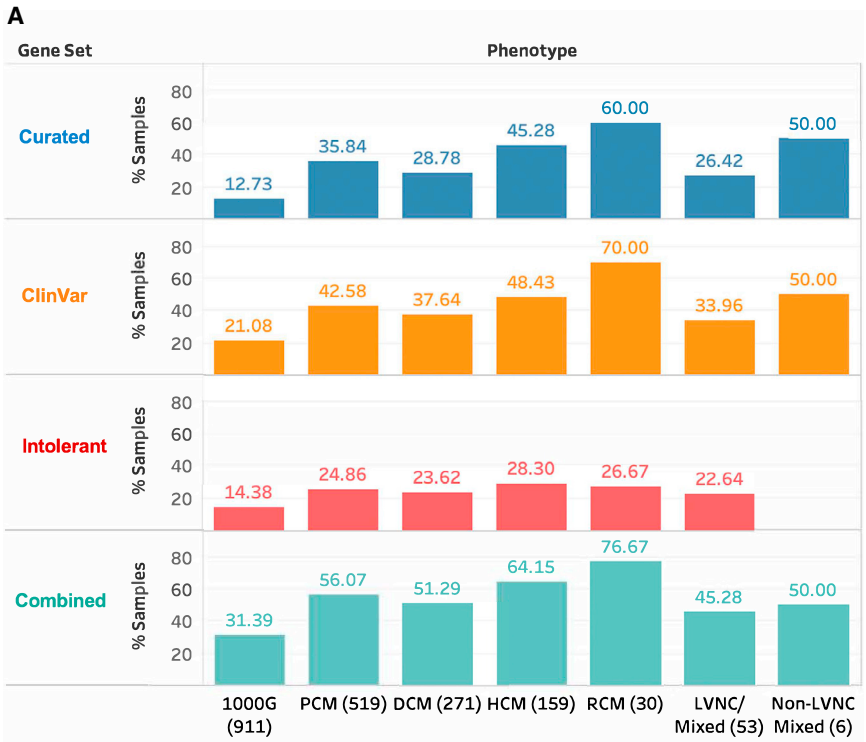


Figure 3. Damaging odds ratios in PCM affected individuals and control individuals

(A) Odds and 95% confidence interval of having a damaging variant in PCM individuals compared to 1000 Genomes control individuals by gene set and ancestry.

(B) Odds and 95% confidence interval of having a damaging variant in PCM individuals compared to secondary control individuals SPARK by gene set and ancestry.

(C) Odds and 95% confidence interval of having a damaging variant in PCM individuals of European ancestry compared to PCM individuals of African ancestry by gene set. The adjusted odds ratios take phenotypic differences into account.



B

Gene Set	Ancestry	1000G (911)	PCM (519)	DCM (271)	HCM (159)	RCM (30)	LVNC/Mixed (53)	Non-LVNC Mixed (6)
		Phenotype						
Curated	EUR*	11.9	40.2	32.1	48.2	58.3	29.2	50.0
	AMR*	13.0	33.6	30.5	37.0	80.0	25.0	50.0
	AFR	18.0	23.5	16.3	38.9	100.0	23.5	0.0
ClinVar	EUR*	19.1	45.8	40.0	50.9	66.7	33.3	50.0
	AMR*	22.5	40.6	36.6	44.4	80.0	41.7	50.0
	AFR	29.5	34.1	32.7	38.9	100.0	29.4	0.0
Intolerant	EUR*	11.1	24.5	23.6	27.2	29.2	16.7	0.0
	AMR	19.6	25.0	19.5	33.3	20.0	50.0	0.0
	AFR	11.5	25.9	30.6	27.8	0.0	11.8	0.0
Combined	EUR*	26.8	58.2	52.9	64.9	75.0	41.7	50.0
	AMR*	37.2	52.3	45.1	63.0	80.0	66.7	50.0
	AFR	36.1	54.1	57.1	61.1	100.0	35.3	0.0

Figure 4. Variant burden analysis in cardiac discovery genes shows enrichment in the pediatric cardiomyopathy cohort

(A) Variant burden, shown as percentage of samples with damaging variant(s), in curated, ClinVar, intolerant, and combined gene sets by phenotype. The percentage is shown above each bar and the number of individuals is shown in parentheses on the x axis.

(B) Variant burden in curated, ClinVar, intolerant, and combined gene sets by ancestry and phenotype. PCM participants are compared to 1000 Genomes (1000G) data. Combined, genes present in curated, ClinVar, missense-intolerant, or LoF-intolerant gene lists. DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LoF, loss of function; LVNC, left ventricular noncompaction; PCM, pediatric cardiomyopathy cohort; RCM, restrictive cardiomyopathy. *Significant difference between PCM and 1000 Genomes (1000G) burden at $p < 0.001$. p values are PCM versus 1000 Genomes as per Wilcoxon rank-sum analysis.

rates across the cardiomyopathy subtypes (Figure 4A) and curated a higher overall burden among all three ancestries (Figure 4B). Among the intolerant gene set (missense-intolerant N = 337 genes; LoF-intolerant N = 457 genes), our approach identified 663 damaging variants, with similar rates across the cardiomyopathy subtypes (Figure 4A). The combined gene set, the union of the curated, ClinVar and intolerant gene sets, revealed that between 45 to 77% of individuals with cardiomyopathy harbored a damaging variant (Figure 4A), with 1,544 unique damaging variants (Table S5). Notably, there was less ancestral variability in the rates than the other gene lists (Figure 4B). For the five genes with the highest frequency of damaging variants in the PCM cohort, we mapped the location of the variants (Figure S6) and performed network analysis of the major interactions (Figure S7). Not surprisingly, damaging variants are localized in major gene domains.

Analyses of the presence or absence of at least one rare variant may miss the impact of multiple damaging hits. To examine this in more detail, we compared the number of individual-level damaging variants in affected individuals to 1000 Genomes control individuals across gene lists, ancestries, and HCM or DCM sub-phenotypes (Figure 5). Multiple damaging variants (more than one per individual) were more common in the PCM cohort than in control individuals, especially for two or three predicted damaging variants, across PCM subtypes (Figure 5). As an additional control for variant burden, we examined the damaging variant burden of 1,000 sets of randomly selected genes (done for each of the four gene lists) and compared it to the burden of damaging variants observed in each of our gene lists (Figure S8). In general, we observe a higher percentage of individuals with zero or singleton variants in random genes compared to selected genes. When comparing these distributions statistically, we find that the upper end of the distribution (75^{th} and 90^{th} centiles) consistently exhibited enrichment for the curated, ClinVar, and cardiac discovery gene lists ($p < 0.0001$, Table S6).

Overall, using this bioinformatic approach, we were able to identify damaging variants in an additional 33% of individuals with pediatric cardiomyopathy (Figure 6). In particular, candidate genes within the intolerant gene set

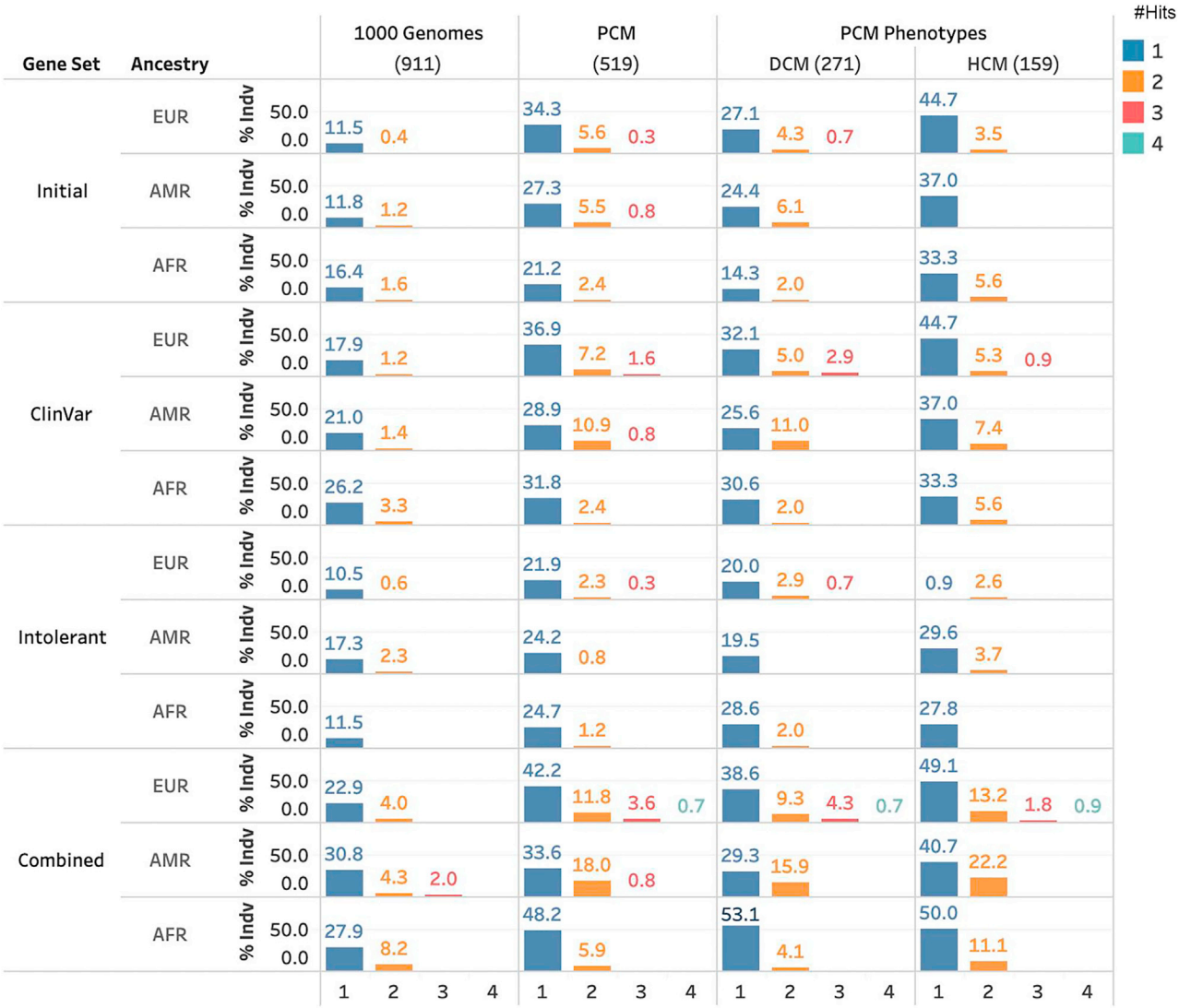


Figure 5. Proportion of individual-level predicted damaging variants by phenotype, ancestry, and gene list
Percentages of one, two, three, or four hits across ancestries (EUR, AMR, and AFR). Combined, genes present in curated, ClinVar, missense-intolerant, or LoF-intolerant gene lists. DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LoF, loss of function; LVNC, left ventricular noncompaction; PCM, pediatric cardiomyopathy cohort; RCM, restrictive cardiomyopathy.

putatively contribute to European ancestry disease for both HCM and DCM and to African American ancestry disease for DCM but do not appear to contribute to admixed American disease in this sample set (Figure 4). Taken together, the results indicate an enriched gene variant burden in PCM participants and suggest that these informatic approaches are useful to delineate additional variants contributing to pediatric cardiomyopathy.

Discussion

While genetic studies have made great strides in identifying the genetic contributions to cardiomyopathy, these studies have largely been restricted to adults or small pediatric studies. Thus, there is a critical need to evaluate the genetic architecture of pediatric onset cardiomyopathy. To address this issue, we assembled a cohort of 528 children with pediatric cardiomyopathy. We found that while clinical genetic testing of children is warranted, continued research is required to expand the number of clinically actionable variants especially for CM subtype, early age of onset, and individuals with non-European ancestry. We came to this conclusion based on the following: (1) 32% of the children had a clinically actionable variant with the most common genes shared between pediatric and adult cases; (2) the rates of clinically actionable variants varied by cardiomyopathy subtype, age of onset, and ancestry; and (3) evidence of rare damaging variant burden, which varied by cardiomyopathy subtype and ancestry.

Clinically actionable variants identified in pediatric CM and impact of sub-phenotype

We found that 32% of pediatric onset cardiomyopathy participants had at least one clinically actionable variant.

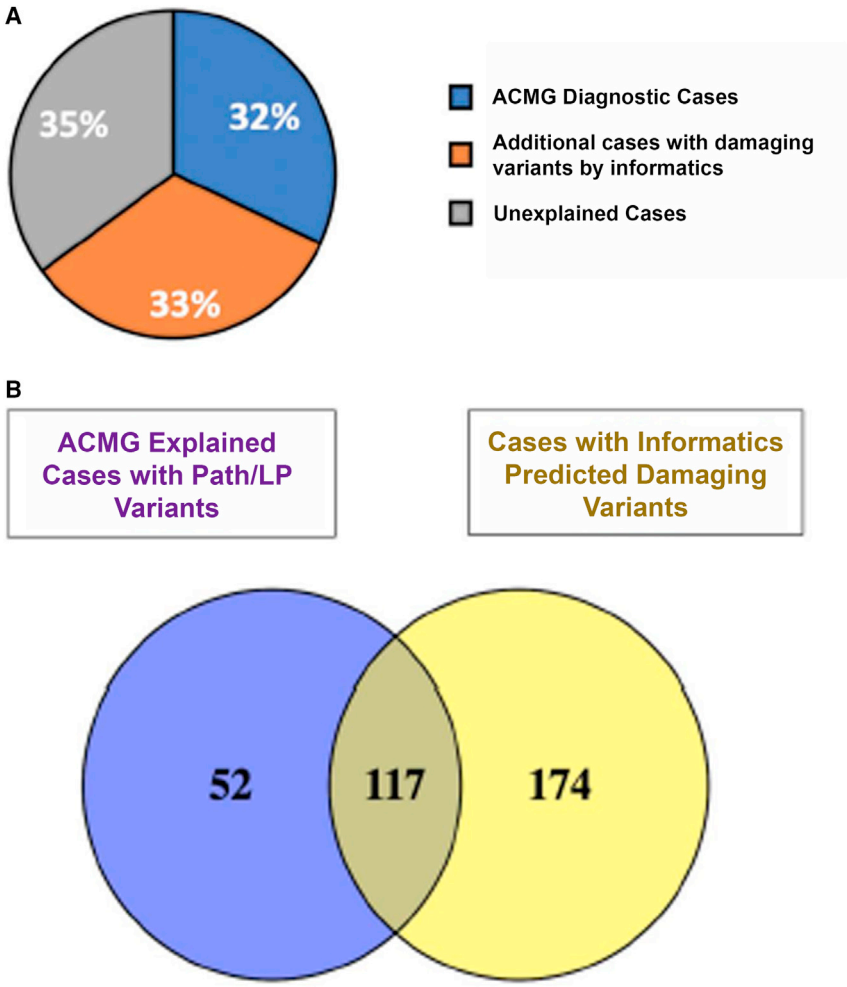


Figure 6. Analysis of cases explained by variants in exomes

(A) Percentage of cases explained with ACMG evaluation and informatic predicted damaging variants.

(B) Venn diagram of cases explained with ACMG evaluation and informatic predicted damaging variants. Values are number of cases.

However, the rates of clinically actionable variants differed markedly by cardiomyopathy subtype, consistent with adult studies.[8,43,44] Identification of molecular disease causation in a family is not always static and it may evolve with changing status of affected individuals in the family and with updates in variant interpretation. Revisiting our variant interpretation dataset of LP/P variants frozen in 2019, we identified ten (7.9%; Table S1) that were downgraded to VUS primarily as a result of updates to ClinVar. Five of these were in *MYH7* based on ClinGen revisions to PM1 and PS4 evidence, reinforcing the recommendations for periodic re-evaluation.[45,46] We note, however, that we were unable to assess or update familial segregation for these variants, evidence which could have confirmed an interpretation of LP. Cardiomyopathy-associated genes continue to be added to ACMG gene list recommendations for return of secondary findings for exome and genome testing.[47] This further supports careful adjudication of variants and the importance of understanding molecular disease causation in the pediatric population.

Most P/LP findings across the cardiomyopathy sub-phenotypes are missense variants; far fewer LoF variants were identified. Notable exceptions include LoF variants in *MYBPC3* for HCM and in *TTN* for DCM. Interestingly, nearly equal numbers of missense and LoF variants in *MYBPC3* were identified in children with HCM. In adults, LoF variants predominate.[48] In children with DCM, only missense variants were found in *MYBPC3*. Although *RBM20* variants have previously been identified as over-represented in children,[11,49] we found only 1.8% of the DCM cohort with P/LP variants, a yield very similar to adults. Overall, HCM and DCM had a similar variant burden. Interestingly, *MYBPC3* variants were not a genetic cause of RCM in our cohort, and *TNNI3* variants explain 30% of cases.

Truncating variants in *TTN* (*TTN*tv) are established causes of DCM and the most commonly identified molecular cause in adults.[29] Age and sex are both known to impact penetrance. In children, reports of *TTN*tv are primarily from smaller studies and vary from ~2%–13% with authors reaching different conclusions about the importance of *TTN* testing in younger children.[22,49–53] Pugh et al. identified causative *TTN* variants in a cohort of children but not infants (age 0–2), and *TTN* variants are the largest cause of DCM in 2–18 year olds.[49] They reported 10% (95% CI, 5.5%–30.6%) P/LP variants in *TTN*, though their sample size was small (n = 29). Another report of DCM in the young showed three of 51 children (6%) with DCM at age < 15 had *TTN* variants, although one affected individual had a congenital heart defect contributing to heart failure. In contrast, *TTN*tv in 15–21 year-olds comprised 23% (7/31) of the affected individuals.[50] The highest yield was found in a recent study of pediatric DCM children in China, with 13% (6/46) children with pathogenic *TTN*tv, with children having an average age of 6.5 years. Of the 279 children with DCM in our study, 3.2% had *TTN*tv, which is at the lower end of previously reported rates. Our variant interpretation focused exclusively on *TTN*tv, so it is possible that some P/LP variants have been missed, though previously published work also often focused on *TTN*tv. Overall, we had a lower familial rate of DCM cases in our cohorts than many studies and this may contribute to a decreased yield. We conclude that *TTN*tv are an important cause of DCM in pre-pubertal children, albeit at frequencies lower than those seen in adults.

This information on genetic test results across pediatric age ranges and cardiomyopathy subtypes adds important evidence for current expert guidelines on the use of genetic testing in children.[12] A positive finding in affected children has important implications for family members who can be risk-stratified by the presence or absence of the familial variant. A diagnostic finding in an affected child provides the opportunity for cost savings and reduced health care use by family members; rather than ongoing cardiac surveillance screening in all first-degree relatives, only those testing positive for the familial variant require surveillance. Future studies could address cost-savings prospectively. Our results most likely represent a minimum estimate, given the number of genes analyzed, although studies have indicated that expanded clinical panels offer limited additional sensitivity, at least for HCM.[11] Finally, it is notable that the mean age of our HCM cohort, which was the oldest sub-phenotype among our cohort, was 11.1 years, highlighting the benefit of early genetic testing and cardiac surveillance and providing strong data to support a more recent recommendation for screening at earlier ages.[12,40,54]

Early age of onset exhibits reduced occurrence of diagnostic molecular findings

We found that nearly 20% of cardiomyopathy affected individuals with diagnosis in infancy had a diagnostic genetic finding as compared to nearly 40% of non-infants. Given that infant presentation was common (36%) at the tertiary centers our study was based on, the lower rate is notable. This trend was consistent across cardiomyopathy sub-phenotypes in the 37 tested genes, suggesting that novel causes remain to be identified in this population. Of note, our study was designed to assess idiopathic and familial cardiomyopathy and children with identifiable syndromic, neuromuscular, or metabolic causes were excluded. However, these presentations can be difficult to ascertain in infants and may be missed. Our assessment of phenocopy genes such as those causing Danon disease, glycogen storage disease resulting from variants in *PRKAG2*, RASopathies, and other causes of pediatric cardiomyopathy identified a small number of cases, and it will be important to assess other metabolic or syndromic causes of disease that might explain additional cases in our infant cohort in future studies. Small studies suggest that multiple pathogenic variants in sarcomeric genes may explain earlier onset of disease in infants,[14,15] but our results do not support this, identifying only 1.3% of individuals (n = 7) with two or more P/LP variants in these well-established cardiomyopathy genes, of which only one was an infant. These data suggest that additional research into the causes of infant-onset cardiomyopathy is needed.

Need for additional gene discovery—Evidence of increased rare variant burden

Using clinical interpretation standards, 32% of PCM children had P/LP variants, suggesting that there is substantial room for improvement, especially given that 36% had a positive family history. Using bioinformatic prediction, the enriched burden seen in our larger gene lists suggests that increasing burden of damaging variants across multiple genes is observed in some cases. We find in our cohort that bioinformatic predictions of damaging variants in candidate cardiomyopathy genes offer the potential to expand yield by up to 33%. While few individuals harbored more than one actionable variant from the curated or ClinVar gene lists, when expanding the gene list, individuals with cardiomyopathy often carry multiple damaging rare variants. Thus, while some cardiomyopathy is inherited in an autosomal dominant manner, it is possible that multigenic inheritance may contribute to some cases. Taken together, these results add to the understanding of the genetic architecture of PCM and indicate opportunities for novel gene discovery.[11]

We used a systems biology method to compile candidate gene lists leveraging the rich amount of information available related to heart function and we used bioinformatic variant effect classification to assess damaging variant burden. Such approaches should be applied with caution, as there is high risk of findings that are not independently causal. Specifically, clinical evaluation of variants based on ACMG guidelines incorporates multiple lines of evidence for each variant. In contrast, the bioinformatic approach performs variant classification by combining variant quality attributes with prediction tools like CADD and Meta-SVM and therefore estimates pathogenicity on the basis of molecular effect. Importantly, our bioinformatic-based prediction of variants exhibited consistency with the P/LP and B/LB calls from clinically interpretation. Using bioinformatics, we also identified damaging variants among those interpreted as VUSs by using clinical evaluation. While a damaging prediction should not be viewed as causal, visualization by heatmap of the ClinVar gene set (Figure 2) highlights gene-phenotype and gene-ancestry specific features. Such analyses may be useful for historically difficult to classify variants such as those in *RYR2* or *SCN5A*. Genes with damaging variants that are over-represented in specific ancestries, such as *MYPBC3* in European or *MYH6* in non-European, require further investigation. Thus, the bioinformatics approach and visualization of burden across a large pediatric cardiomyopathy cohort may provide important information to help guide molecular genetic classification of variants.

Need for diversity inclusion: Differences by ancestry

Using a bioinformatic approach to assess the burden of damaging rare variants in known cardiomyopathy genes and candidate genes, we found that individuals of European descent had increased damaging variant burden as compared to individuals of admixed American or African American ancestry. Most gene discovery efforts have been completed in European descent populations and

failure to use ancestry-matched controls can result in misinterpretation of variants.[55] Analyses of diverse biobanks have also identified that participants of African ancestry had more VUSs in the 30 ACMG cardiac actionable genes than those of European ancestry.[56] Our data support shared genetic influences across ancestral groups while also suggesting that some causal variants may be ancestry specific. Notably, we found that damaging variants in *MYBPC3* were absent among individuals of African American ancestry across all sub-phenotypes, and although our numbers of African American children with HCM were small (n = 18), the result was statistically significant. A study of South Africans reported a markedly lower genetic diagnosis rate for HCM (29%), although the study size was small.[57] As *MYBPC3* accounted for 23% of our HCM cohort, these results support the premise that undiscovered genes contribute to PCM in individuals of African descent. It is also possible that there are modifier loci whose frequencies vary by ancestry. Indeed, two recent investigations highlight genetic variants in *BAG3* in African Americans (but not Europeans) with cardiomyopathy that modify heart failure outcomes.[58,59] With the goal of identifying candidate genes, we created our intolerant gene set. In contrast to the curated and ClinVar gene sets, in which children of European ancestry were more likely to harbor damaging variants than those of African ancestry, using the intolerant gene set, individuals of African ancestry had similar damaging variant burden. Thus, these genes represent plausible candidates for ancestry-specific or ancestry-enriched variant burden. Most of the intolerant genes with burden in the PCM exomes (N = 201 genes) were enriched in abnormal heart morphology genes as per mouse models (66.1%, 133/201 genes, $p < 0.001$) and human heart development (42.3%, 85/201 genes, $p < 0.001$). Few of these genes were enriched in sarcomere cellular component (14.4%, 29/201 genes, $p < 0.001$) or in myofibrils (14.4%, 29/201 genes, $p < 0.001$). Only 13 of 201 intolerant genes were in the ClinVar gene set. These results highlight the need for gene discovery efforts on diverse populations.

Conclusion

In summary, this multi-site study of primary PCM children in North America demonstrates that while the genes identified from the primarily adult genetic studies on cardiomyopathy provide clinical value, there is room for improvement. Specifically, our results suggest that the expansion of more diverse study populations including infants and individuals with non-European ancestry will be essential to improve diagnostic genetic testing yields. Further, the demonstration of increased rare damaging variant burden suggests the need to consider additional genes as well as multigenic inheritance models. We expect that this large pediatric cohort will provide pediatricians the evidence required to support broad clinical genetic testing and researchers' opportunities to examine novel genetic contributions.

Data and code availability

The dataset generated during this study are in preparation for submission to dbGaP (https://www.ncbi.nlm.nih.gov/gap/) and will be added to the manuscript when available.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.ajhg.2021.12.006.

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Declaration of interests

J.W.R. is a consultant for Amgen, Bayer, Novartis, and Abiomed. W.K.C. is on the scientific advisory board for the Regeneron Genetics Center. S.E.L. is a consultant for Tenaya Therapeutics and Bayer and on an advisory board for Myokardia.

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Web resources

1000 Genomes, https://www.internationalgenome.org/data/
CADD, https://cadd.gs.washington.edu
ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/
ExAC/pLi, http://exac.broadinstitute.org
GATK 3.3, https://gatk.broadinstitute.org/hc/en-us
GeneReviews, Hershberger, R.E., and Jordan, E. (2007). Dilated Cardiomyopathy Overview, https://www.ncbi.nlm.nih.gov/books/NBK1309/
gnomAD, https://gnomad.broadinstitute.org
JMP 14.0, https://www.jmp.com/en_us/home.html
OMIM, https://omim.org/
Plink, https://www.cog-genomics.org/plink/
python 2.7, https://www.python.org
R 4.0.1, https://www.r-project.org
ToppGene Suite, https://toppgene.cchmc.org/
VEPv97/dbNSFP/MetaSVM, https://useast.ensembl.org/info/docs/tools/vep/index.html

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