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ORIGINAL ARTICLE

Variant Interpretation for Dilated Cardiomyopathy

Refinement of the American College of Medical Genetics and Genomics/ ClinGen Guidelines for the DCM Precision Medicine Study

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BACKGROUND: The hypothesis of the Dilated Cardiomyopathy Precision Medicine Study is that most dilated cardiomyopathy has a genetic basis. The study returns results to probands and, when indicated, to relatives. While both the American College of Medical Genetics and Genomics/Association for Molecular Pathology and ClinGen's *MYH7*-cardiomyopathy specifications provide relevant guidance for variant interpretation, further gene- and disease-specific considerations were required for dilated cardiomyopathy. To this end, we tailored the ClinGen *MYH7*-cardiomyopathy variant interpretation framework; the specifications implemented for the study are presented here.

METHODS: Modifications were created and approved by an external Variant Adjudication Oversight Committee. After a pilot using 81 probands, further adjustments were made, resulting in 27 criteria (9 modifications of the ClinGen *MYH7* framework and reintroduction of 2 American College of Medical Genetics and Genomics/Association of Molecular Pathology criteria that were deemed not applicable by the ClinGen *MYH7* working group).

RESULTS: These criteria were applied to 2059 variants in a test set of 97 probands. Variants were classified as benign (n=1702), likely benign (n=33), uncertain significance (n=71), likely pathogenic (likely pathogenic; n=12), and pathogenic (P; n=3). Only 2/15 likely pathogenic/P variants were identified in Non-Hispanic African ancestry probands.

CONCLUSIONS: We tailored the ClinGen *MYH7* criteria for our study. Our preliminary data show that 15/97 (15.5%) probands have likely pathogenic/P variants, most of which were identified in probands of Non-Hispanic European ancestry. We anticipate continued evolution of our approach, one that will be informed by new insights on variant interpretation and a greater understanding of the genetic architecture of dilated cardiomyopathy.

CLINICAL TRIAL REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT03037632

Key Words: cardiomyopathy, dilated ■ genetics ■ genetic testing ■ genomics ■ pathology, molecular

The Dilated Cardiomyopathy (DCM) Precision Medicine Study was launched in 2015 to test the hypothesis that both familial and nonfamilial DCM have a genetic basis. For this study, DCM is defined as nonischemic disease and idiopathic, after excluding nongenetic etiologies.[1] The study, which aims to recruit 1300 individuals with DCM (600 non-Hispanic ethnicity African Ancestry, 600 non-Hispanic ethnicity-European Ancestry, and 100 Hispanic ethnicity; 50% female) and 2600 relatives, includes a randomized controlled trial to evaluate the effectiveness of an educational guide (Family Heart Talk) on the uptake of and adherence to cardiovascular screening in at-risk relatives. Families are recruited at DCM Consortium sites, by telephone, and mail.[2]

After exome sequencing in probands and affected relatives, results from analysis of 35 DCM genes (see

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*A list of all DCM Consortium participants is given in the Appendix.

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Nonstandard Abbreviations and Acronyms

ACMG	American College of Medical Genetics and Genomics
DCM	dilated cardiomyopathy
LOF	loss of function
LP	likely pathogenic
P	pathogenic
VUS	variant of uncertain significance

Data Supplement) are returned to probands and relatives of probands who are found to carry likely pathogenic or pathogenic (LP/P) variants. As part of the study, periodic screening is recommended for unaffected relatives with LP/P variants. Unaffected relatives who do not carry a known LP or P variant identified in the proband are discharged from surveillance. For at-risk relatives of probands in whom a LP/P variant is not identified, surveillance is recommended. These screening recommendations follow Heart Failure Society of America and the American College of Medical Genetics and Genomics (ACMG)[3,4] guidelines and depend on accurate variant interpretation.

The ability to conduct large-scale molecular genetic testing has resulted in an increased volume of sequence variants, and, consequently, new challenges in interpretation. This prompted ACMG to revise prior guidance,[5] resulting in updated standards, jointly with the AMP (Association for Molecular Pathology).[6] Recognizing that the ACMG/AMP standards serve a broad spectrum of single-gene conditions, the ClinGen[7] Cardiovascular Domain Working Group tailored ACMG/AMP guidance for cardiovascular disease.[8] Incorporating the genetic features of cardiomyopathy (phenotypic, locus, and allelic heterogeneity, reduced and adult onset penetrance, multiple variants interacting to cause disease), the ClinGen Inherited Cardiomyopathy Expert Panel (CMP-EP), which included 2 members of this author group (A. Morales, Dr Hershberger), published specifications for hypertrophic and dilated cardiomyopathies focused on *MYH7*.[9] The *MYH7* specifications provided a foundation for the variant interpretation criteria needed to conduct our study; however, further specifications were required to incorporate considerations that are unique to DCM. To this end, we tailored the ClinGen *MYH7* variant interpretation framework. The resulting criteria, along with preliminary data, are presented here.

METHODS

Please see the Data Supplement for complete methods. The relevant variant curation criteria are summarized (Table 1). The nonpilot variant data used for this analysis, excluding 1500 unique variants unlikely to change protein function based on predicted Sequence Ontology[10] impacts, are provided in the Data Supplement. Source code and complete variant and adjudication data are available upon request from the authors. All research participants provided signed informed consent. Institutional Review Board approval was obtained for this study, initially locally at each of the participating clinical sites of the DCM Consortium, and later with study oversight provided by a single Institutional Review Board at the University of Pennsylvania.

RESULTS

Pilot Study

A pilot study was conducted with the first 81 probands enrolled in the study. Of these, 40 (49%) carried only likely benign (LB) or benign (B) variants. The rest carried variants of uncertain significance (VUS; n=30, 37%) and LP variants (n=11, 14%). Three challenges were identified from this exercise: limited gene-specifications for predicted loss-of-function (LOF) variants, evaluation of a variant's absence or rarity in reference populations, and use of computational data. Further adjustments, as described below, were approved by the Variant Adjudication Oversight Committee (VAOC; Chair, Dr Jarvik; Members, Drs Burke, Dorschner, Gastier-Foster, and Rehm).

To address the first challenge, a very strong criterion for pathogenicity for LOF variants in *LMNA* and *SCN5A* (PVS1) and a strong criterion for pathogenicity for LOF variants in *FLNC, BAG3,* and *TTN* A-band (PVS1_Strong) were added. The moderate criterion (PVS1_Moderate) was applied to LOF variants in *VCL, PLN,* and *DSP*. These modified criteria were applied considering available evidence associated with DCM. LOF has been established as a disease mechanism for *LMNA*-DCM and other *LMNA*-associated diseases.[11] Supporting this are large multicenter studies demonstrating a substantially increased odds ratio in DCM cases versus controls (odds ratio=99.7).[12] Furthermore, in the latest release of gnomAD (v2.1.1), the *LMNA* pLI (probability of being loss-of-function intolerant) was established to be 1.00. For *SCN5A*, LOF variants are pathogenic for Brugada syndrome.[13,14] Also, a large multicenter study demonstrated a significantly increased odds ratio of 16.5 for LOF variants in *SCN5A* in DCM cases versus controls.[12] In addition, the p.Arg219His variant, identified in DCM and arrhythmias,[15] was studied in *Xenopus* oocytes, leading to a proton leak but not sodium current alterations. While no segregation has been demonstrated for LOF in primary DCM, supporting segregation data have been published in a family with sick sinus syndrome, and studies in the human cell line tsA-201 with heterologously expressed mutant sodium channels showed LOF properties of reduced or no sodium current density in conjunction with gating modulations.[16] For this gene, the gnomAD pLI score is 0.91. We also note that when a truncating

Table 1. DCM Precision Medicine Study Variant Interpretation Criteria

Strength	Specification Type	Rule Abbreviation	Rule Description
Pathogenic criteria			
Very strong	Reintroduced ACMG criterion for DCM gene specification	PVS1	Null variant in *LMNA* or *SCN5A*
Strong	None	PS1	Same amino acid change as a previously established pathogenic variant
	None	PS2	De novo (paternity confirmed) in a patient with disease and no family history
	None	PS3	Well-established in vitro or in vivo functional studies supportive of a damaging effect
	None	PS4	Prevalence of variant in affected individuals is significantly increased compared with controls OR variant identified in ≥15 probands with consistent phenotypes OR variant identified in ≥10 confirmed unlreated probands with consistent phenotypes
	None	PP1_Strong	Variant segregates in ≥7 meioses in DCM genes with established evidence
	Strength modification, DCM gene specification	PVS1_Strong	Null variant in *FLNC*, *BAG3,* or *TTN* A-band
Moderate	DCM gene specification	PM1	Located in hotspot (For DCM, *RBM20* exon 9, amino acids 634, 636, 637, 638)
	Method specification, DCM gene specification, study specific	PM2	Absent from gnomAD or at extremely low frequency in all gnomAD nonfounder populations with available data
	Reintroduced ACMG criterion, DCM gene specification	PM3	For recessive disorders, detected in trans with a pathogenic variant (rarely observed with *TNNI3* and *TNNT2* variants)
	None	PM4	In-frame deletions/insertions of any size in a nonrepeat region or stop-loss variants
	None	PM5	Different missense change at same amino acid residue previously established as pathogenic
	DCM specific	PM6	De novo without confirmation of paternity (parental clinical data required)
	DCM gene specification	PVS1 _Moderate	Null variant in *VCL, PLN,* or *DSP*
	None	PS4 _Moderate	Variant identified in ≥6 probands with consistent phenotypes
	None	PP1 _Moderate	Variant segregates in ≥5 meioses in DCM genes with established evidence
Supporting	None	PP1	Variant segregates in ≥3 meioses in DCM genes with established evidence
	Method specification	PP3	Multiple lines of computational evidence support a deleterious effect (REVEL score >0.7)
	None	PS4 _Supporting	Variant identified in ≥2 probands with consistent phenotypes
Benign			
Stand alone	Method specification	BA1	Variant allele frequency is >0.1% in any gnomaAD nonfounder population
	DCM gene specification	BP1_Stand _Alone	Missense variant in *TTN*
Strong	Method specification, DCM gene specification, study specific	BS1	Variant allele frequency is >0.05% in any gnomAD nonfounder population
	None	BS3	Well established in vitro or in vivo functional studies show no damaging effect
	None	BS4	Nonsegregation in affected members of a family
Supporting	None	BP2	Observed as a compound heterozygote (in trans) or double heterozygote in genes with overlapping function
	Method specification	BP4	Multiple lines of computational evidence suggest no impact on gene or gene product (REVEL score <0.15)
	Study specific	BP7	Unlikely to affect protein function based on calculated Sequence Ontology terms for the transcript(s) of interest

ACMG indicates American College of Medical Genetics and Genomics; and DCM, dilated cardiomyopathy.

SCN5A variant is identified in a proband with DCM, a search for an arrhythmia phenotype is also warranted.

LOF variants in *FLNC, BAG3*, and the A-band of *TTN* were assigned strong criteria for pathogenicity. First, for *FLNC*, a LOF mechanism has been reported[17] and the gnomAD pLI=1.00. Due to the relative gene size of *FLNC* and *LMNA*, the pLI calculation appears to be reliable. However, in smaller genes, where variants are less prevalent

in the DCM population although absent from controls, the pLI may not reflect the actual contribution of LOF variants to disease. For *BAG3*, a gene comprising 4 coding exons, despite LOF been known as a disease mechanism,[18,19] the gnomAD pLI=0.62. In the case of *TTN*, the coding sequence leads to the generation of alternative splicing isoforms,[20] with the heart expressing 2 major isoforms (N2B from transcript NM_003319.4 and N2BA from transcript NM_001256850.1) that incorporate the Z-line, I-band, A-band, and M-line.[20] The N2B and N2BA isoforms diverge mainly for the incorporation in the long-cardiac isoform N2BA of a large portion of I-band coding exons, similar to the skeletal muscle isoform N2A derived from transcript NM_133378.4.[20] It is known that LOF variants affecting the *TTN* A-band (defined as chr2:179483218-179400709, NM_001267550.1, GRCh37/hg19) are overrepresented in DCM,[21,22] establishing LOF as a mechanism of disease.[12] However, due its large size, potential LOF variants are differently distributed across the various functional domains between DCM cases and controls.[23] The high prevalence in the control population of LOF variants in *TTN* regions other than the A-band led to a calculated gnomAD pLI=0.00; however, the calculation does not discriminate between the various transcripts and protein domains and thus does not accurately reflect the deleterious role of LOF variants in the A-band.[24]

LOF variants in *VCL, PLN,* and *DSP* were assigned a moderate degree of pathogenicity, although for a *DSP* variant in this category, the variant must have been observed in at least one unrelated DCM case. We implemented this criterion for *VCL* and *DSP* LOF variants based on their enrichment in DCM cases versus controls (OR_{VCL}=21.3; OR_{DSP}=41.0).[12] In addition, the constraint metrics for *DSP* support a LOF mechanism (pLI=1.00). For *PLN*, a trend for enrichment of LOF variants was observed in DCM cases versus controls (OR_{PLN}=13.8).[12] In addition, *PLN* LOF variants have been shown to be pathogenic and described in both DCM and HCM.[25–29] For both *PLN* and *VCL*, the constraint metrics from gnomAD are below threshold (*PLN*, pLI=0.45; *VCL* pLI=0.05); however, the *PLN* coding sequence has only one exon, possibly being too small a target to reliably detect population levels of LOF variants. Haploinsufficiency of *VCL*, although enriched in DCM, may represent a low penetrant event. However, we suggest that additional data are needed to confirm our preliminary *VCL* observations for LOF.

When evaluating PM2, Wilson's score CIs could not be calculated if there were no genotype calls at the variant position in both exomes and genomes, which could occur due to either the absence of the variant in all populations or lack of coverage. To allow using PM2 in the former scenario, the criterion was adjusted so it was met if the allele count was sufficient (120 000) in neighboring nucleotides, coverage was sufficient, and a mix of exome and genome data was available in gnomAD.

Evaluation of computational data (PP3 and BP4) was performed manually for this pilot using a holistic approach. To harmonize computational data evaluation, a method based on the REVEL score[30] was introduced. Cutoffs were developed for this study on the basis of the publication (which estimated the sensitivity and specificity of each potential cutoff using out-of-bag predictions for 6182 HGMD disease mutations and 123 706 rare putatively neutral exome sequence variants)[30] and made these data publicly available (https://sites.google.com/site/revelgenomics/). On the basis of these data, variants meeting BP4 must have a REVEL score below 0.15, a cutoff estimated to falsely predict no deleterious effect for ≈5% of pathogenic variants while correctly identifying ≈55% of neutral variants (Figure). Variants meeting PP3 must have a REVEL score >0.7, which would correctly identify ≈58% of pathogenic variants and falsely predict a deleterious effect for only ≈5% of neutral variants. When comparing calls made before REVEL implementation to those that would have been made after, we found that 5/6 variants <0.15 cutoff had been manually classified as meeting BP4. Among the 13 variants that had a REVEL score >0.7, 12 had been previously classified as meeting PP3. Of 26 variants with a REVEL score between 0.15 and 0.7, 4 had been manually classified as meeting BP4, 14 met PP3, and 8 met neither. Although variants with a score between 0.15 and 0.7 would not receive an automatic PP3 or BP4 evaluation using the REVEL method, manual review of the data and clinical judgment can be applied if application of the PP3 or BP4 criteria would lead to a clinically significant difference in variant classification.

RESULTS USING DCM VARIANT INTERPRETATION CRITERIA ADAPTATIONS

Our modifications resulted in 27 criteria for variant interpretation in DCM as summarized (Table 1); full documentation is also provided (Data Supplement). Using this framework, curation was performed between August 2018 and April 2019 for 2059 variants in an additional set of 97 probands (Data Supplement). Variants were classified as B (n=1702), LB (n=33), VUS (n=71), LP (n=12), and P (n=3). Two hundred sixteen (10%) were not adjudicated (low impact variants for which BP7 was met); 22 (1%) were classified as low quality.

Among the 97 probands, P variants were identified in 3%, LP in 12%, and VUS in 46%, (Table 2). Most LP/P variants were in *TTN* (n=8; Table 3). Variants reached LP/P classifications based on case (n=1, PS4; n=3, PS4_Moderate; n=3, PS4_Supporting) and segregation data (n=2, PP1_Strong; n=2, PP1), allele frequency (n=15, PM2), predicted loss of function (n=1, PVS1; n=11, PVS1_Strong), protein length change (n=1,

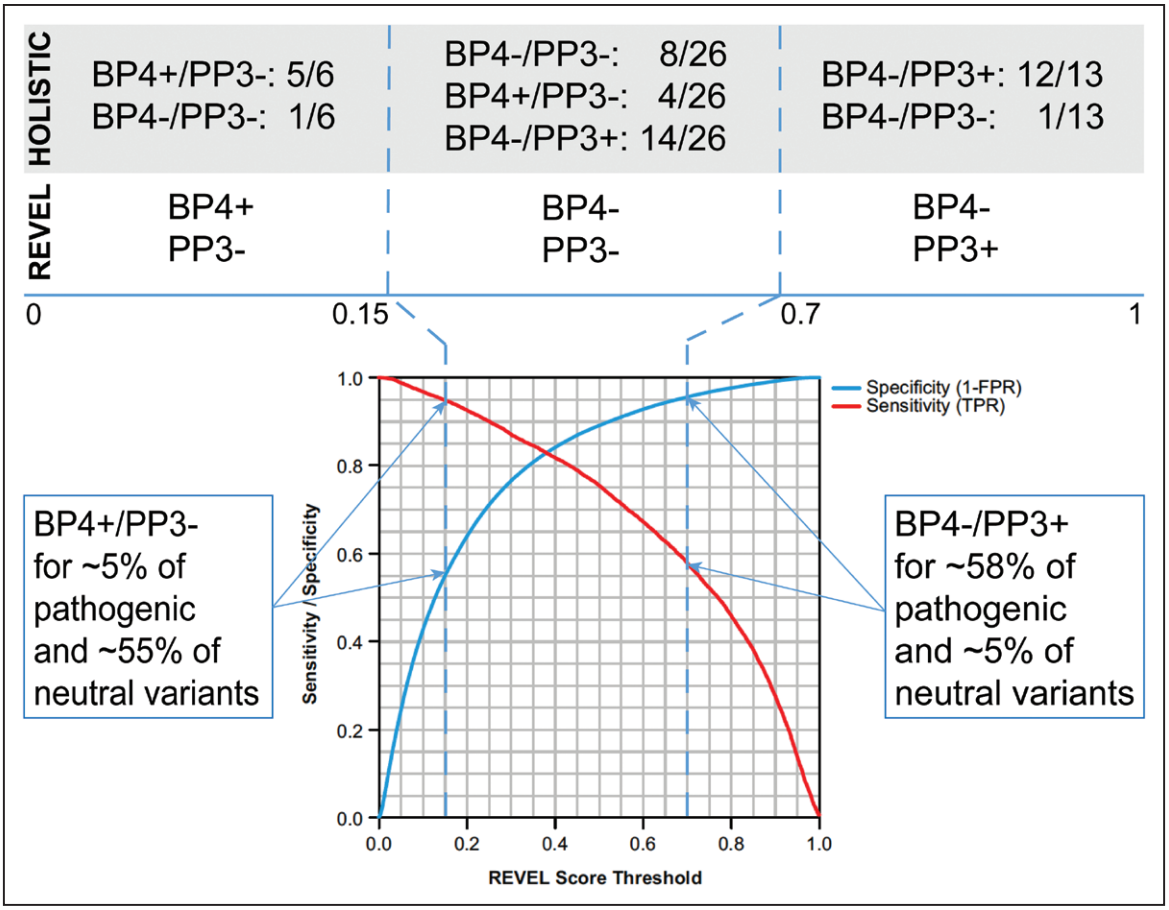

Figure. Automated REVEL score utilization.
Cutoffs for BP4/PP3 were based on the estimated sensitivity and specificity reported in Figure S1 in the Data Supplement of Ioannidis et al.[30] Comparison of holistic evaluations of variants in the pilot phase with automatic evaluations for the same variants based on the selected REVEL score cutoffs are shown above the blue line. Reprinted from Ioannidis et al with permission. Copyright 2016 ©, Elsevier.

PM4), hotspot location (n=1, PM1), and computational evidence (n=2, PP3). LP/P variant types included stop gained (n=6/15, 40%), frameshift (n=6/15, 40%), in-frame deletion (n=1/15, 7%), and missense (n=2/15, 13%). Of the 15 LP/P variants, 13 were found in individuals of non-Hispanic European ancestry. More than one VUS and above variant were identified in 20 (21%) probands; however, combinations including more than one LP/P variant were not observed in this preliminary sample.

DISCUSSION

We tailored the ACMG and ClinGen *MYH7* variant interpretation criteria to DCM. Previous publications report a ≈27% detection rate for LP/P variants in DCM[31]; however, the detection rate for LP/P variants using our criteria so far has only been 15.5%. A significant proportion of these variants are predicted LOF. Significant due diligence was done when applying PVS1 rules for this study. Variants affecting the initiation codon were not automatically assumed to cause

Table 2. Most Severe Variant Among 97 Probands With DCM, by Ethnicity and Ancestry

	Benign/ Likely Benign	Uncertain Signficance	Likely Pathogenic	Pathogenic	Total
	N (Row %)				
Non-Hispanic, White	14 (29.2%)	21 (43.8%)	10 (20.8%)	3 (6.3%)	48
Hispanic, White	0 (0%)	2 (100%)	0 (0%)	0 (0%)	2
Non-Hispanic, African Ancestry	23 (48.9%)	22 (46.8%)	2 (4.3%)	0 (0%)	47
Hispanic, African Ancestry	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0
Total	37 (38.1%)	45 (46.4%)	12 (12.4%)	3 (3.1%)	97
Row percentages may not add to exactly 100% due to rounding					

DCM indicates dilated cardiomyopathy.

Table 3. Variant Details for Likely Pathogenic and Pathogenic Variants, by Ethnicity and Ancestry

Adjudication	Ethnicity, Ancestry	Gene	HGVSc	HGVSp	Impacts
Pathogenic	Non-Hispanic, White	LMNA	NM_170707.3:c.673C>T	NP_733821.1:p.Arg225Ter	Stop gained
		TNNT2	NM_000364.3:c.650_652delAGA NM_001001430.2:c.629_631delAGA	NP_000355.2:p.Lys217del NP_001001430.1:p.Lys210del	Inframe deletion
		TTN	NM_001267550.1:c.61876C>T NM_133378.4:c.54172C>T	NP_001254479.1:p.Arg20626Ter NP_596869.4:p.Arg18058Ter	Stop gained
Likely pathogenic	Non-Hispanic, White	BAG3	NM_004281.3:c.1417C>T	NP_004272.2:p.Arg473Ter	Stop gained
		FLNC	NM_001458.4:c.4926_4927insACGTCACA	NP_001449.3:p.Val1643ThrfsTer26	Frameshift variant
		FLNC	NM_001458.4:c.1948C>T	NP_001449.3:p.Arg650Ter	Stop gained
		LMNA	NM_170707.3:c.949G>A	NP_733821.1:p.Glu317Lys	Missense variant
		RBM20	NM_001134363.1:c.1906C>T	NP_001127835.1:p.Arg636Cys	Missense variant
		TTN	NM_001267550.1:c.90844delA NM_133378.4:c.83140delA	NP_001254479.1:p.Thr30282ArgfsTer8 NP_596869.4:p.Thr27714ArgfsTer8	Frameshift variant
		TTN	NM_001267550.1:c.88703_88704delAC NM_133378.4:c.80999_81000delAC	NP_001254479.1:p.His29568LeufsTer7 NP_596869.4:p.His27000LeufsTer7	Frameshift variant
		TTN	NM_001267550.1:c.66804_66807delGAAG NM_133378.4:c.59100_59103delGAAG	NP_001254479.1:p.Lys22269HisfsTer10 NP_596869.4:p.Lys19701HisfsTer10	Frameshift variant
		TTN	NM_001267550.1:c.64915C>T NM_133378.4:c.57211C>T	NP_001254479.1:p.Arg21639Ter NP_596869.4:p.Arg19071Ter	Stop gained
		TTN	NM_001267550.1:c.71307_71310dupTGAC NM_133378.4:c.63603_63606dupTGAC	NP_001254479.1:p.Ser23771Ter NP_596869.4:p.Ser21203Ter	Frameshift variant
	Non-Hispanic, African Ancestry	TTN	NM_001267550.1:c.83515C>T NM_133378.4:c.75811C>T	NP_001254479.1:p.Arg27839Ter NP_596869.4:p.Arg25271Ter	Stop gained
		TTN	NM_001267550.1:c.66931_66932delAA NM_133378.4:c.59227_59228delAA	NP_001254479.1:p.Lys22311ValfsTer3 NP_596869.4:p.Lys19743ValfsTer3	Frameshift variant

haploinsufficiency due to the possible rescue mechanism of a downstream methionine. Also, variants predicted to lead to premature termination codon or predicted to affect mRNA stability occurring in the last exon and last 55nt of the second-last exon possibly escaping nonsense mediated decay were excluded from PVS1 application. Similarly, invariant splice site variants would be considered as leading to a null variant only if a new in-frame splice junction is not predicted and the variant affects all biologically functional transcripts known to be active in the heart and altered in DCM. These principles are consistent with published guidance for interpretation of LOF variants.[32]

Compared with a decade ago, concluding pathogenicity in Mendelian disorders requires a higher level of evidence.[33] This approach strengthens the degree of certainty when reporting variants. While our approach adds gene-specificity, a limitation not unique to this study is that variant adjudication is a probabilistic process. Achieving precision medicine in DCM requires large data sets and functional studies that demonstrate pathogenicity. Further evaluation of these criteria by an independent group, such as the ClinGen CMP-EP, could serve the community by generating international consensus on variant interpretation for DCM.

With continued use of our criteria and an ever-expanding literature body on variant interpretation, cases will emerge that provide opportunities to refine this framework. For example, we recognize that restricting pathogenicity to the A-band of *TTN* excludes potentially relevant regions that can be identified with more granular analyses.[34,35] We have the infrastructure to support ongoing, thoughtful reanalysis and refinement of our criteria. At study completion, a re-evaluation will be undertaken, incorporating best practices and any new data. In addition, there is potential for reassessment of low-quality variants and those that were not adjudicated. Variant reclassifications may result from this process, and, following published principles for researchers returning results,[36] should this occur while the study is active, our protocol has provisions for re-contacting participants.

DCM genetic testing results almost always impact families. In probands, LP/P results may directly affect treatment. For example, DCM patients with LP/P variants in arrhythmogenic genes (eg, *LMNA*, *FLNC*) should have an implantable cardioverter defibrillator placed earlier.[3] Negative testing results neither exclude the possibility that the proband's condition has a genetic etiology nor relieve relatives from risk.

Variant reclassifications also have an impact. The highest-impact reclassifications involve downgrading of LP/P variants. In this scenario, where at-risk relatives would have been offered cascade testing, those found not to carry the variant would have been informed in error that no additional cardiac surveillance is recommended. We note, however, that in the case of LP variants, where a 10% error rate is assumed,[6] participants in this category are informed that no additional

surveillance is needed beyond what would routinely be recommended for the general population. The counseling conversation also includes discussion of symptoms that should be reported to their physician. Despite these caveats, relatives who do not carry a familial LP or P variant may be discharged from surveillance. When instead of downgraded, variants are upgraded to LP/P, cascade testing is made possible, without which at-risk relatives would have had only the option of cardiac screening. Reclassifications involving changes to and from LB/B and VUS have less clinical impact: cardiac screening should continue in at-risk relatives in every case.[3,4]

Although we prefer a conservative approach for return of results in this study, understanding the genetic architecture of DCM will require substantial further research. Indeed, a proportion of DCM may be oligogenic,[37] but the proportion following such a mechanism remains uncertain. We note that a high rate of VUSs in *BRCA* genes were reclassified as LB or B, although in that study only 6.4% (2868 of 44 777) of unique variants were reclassified[38]; whether these data predict a similar eventual resolution of VUSs observed in DCM also remains uncertain. Augmented methods for variant interpretation allowing the assessment of criteria consistent with oligogenic inheritance will be needed for disorders with complex genetics, including DCM. Such methods may provide a pathway to evaluate variants that currently do not exceed a VUS classification.

Cohort diversity is essential to understanding the genetic architecture of complex inherited disorders,[39] including DCM. Our study aims to evaluate the genetic foundation of DCM, regardless of family history, racial, or ethnic background. Our sample of 97 probands included 47 individuals of Non-Hispanic, African Ancestry; actionable variants were identified in only 2 of these participants (4.3% versus 27.1% in those of Non-Hispanic European ancestry). Although a variant may reach a pathogenic classification based on data from a single family, in reality most pathogenic variants, particularly missense variants, reach such classification only after combining evidence from multiple families and resources. Our study is contributing to the evidence base needed to adjudicate variants in non-European ancestry populations, but at the same time depends on currently existing evidence to classify variants in individuals of diverse backgrounds. Although we present these preliminary results for the purpose of detailing the development and performance of our variant adjudication and return of results framework, our findings echo the call for ongoing attention to and inclusion of non-European ancestry families in genetics research.[40,41]

CONCLUSIONS

The DCM Precision Medicine Study has developed an approach to variant classification by adapting ACMG/AMP and ClinGen guidelines. Following modifications that were deemed necessary after a pilot study of 81 probands, the new framework was applied to a test set of 97 probands, and locked for continued use in this DCM research study. Our preliminary data show that 15.5% of DCM probands had LP/P variants, and most of these were identified in individuals of Non-Hispanic European ancestry. Our results highlight the value of ethnically and racially diverse family units in research, but also illustrate the challenges in variant interpretation for genetically complex disorders.

ARTICLE INFORMATION

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Disclosures

None.

APPENDIX

DCM Consortium institutions and personnel participating in this study: Study Principal Investigator and Co-Investigators: The Ohio State University: Ray E. Hershberger (Principal Investigator), Daniel D. Kinnamon, Ana Morales, Elizabeth Jordan; Nationwide Children's Hospital: Julie M. Gastier-Foster; University of Washington: Wylie Burke, Deborah J. Bowen, Deborah A. Nickerson, Michael 0. Dorschner. DCM Consortium Clinical Site Principal Investigators and Clinical Site Other Significant Contributors (OSC). The following clinical sites and individuals contributed to the submission of RO 1 H L 128857 as Site Principal Investigators (Site PI) or as Other Significant Contributors (OSC); The Ohio State University: Garrie Haas (Site PI), William T. Abraham (OSC), Philip F. Binkley (OSC), Ayesha Hasan (OSC), Jennifer Host (OSC), Brent Lampert (OSC), Sakima Smith (OSC); Tufts University School of Medicine: Gordon S. Huggins (Site PI), Dr. Huggins also served as study co-principal investigator; David D. DeNofrio (OSC), Michael Kiernan (OSC); University of Washington: Daniel Fishbein (Site PI), Richard Cheng (OSC), Todd Dardas (OSC), Wayne Levy (OSC), Claudius Mahr (OSC), Sofia Masri (OSC), April Stempien-Otero (OSC); University of Maryland: Stephen S. Gottlieb (Ste PI); Stanford University Medical Center: Matthew Wheeler (Site PI), Euan Ashley (OSC); Medstar Health Research Institute, Washington Hospital Center: Mark Hofmeyer

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