

# Prospective Evaluation of the Utility of Whole Exome Sequencing in Dilated Cardiomyopathy

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**Background**—Dilated cardiomyopathy may be heritable but shows extensive genetic heterogeneity. The utility of whole exome sequencing as a first-line genetic test for patients with dilated cardiomyopathy in a contemporary “real-world” setting has not been specifically established. Using whole exome sequencing with rigorous, evidence-based variant interpretation, we aimed to identify the prevalence of a molecular diagnosis in patients with dilated cardiomyopathy in a clinical setting.

**Methods and Results**—Whole exome sequencing was performed in eligible patients (n=83) with idiopathic or familial dilated cardiomyopathy. Variants were prioritized for curation in up to 247 genes and classified using American College of Medical Genetics and Genomics–based criteria. Ten (12%) had a pathogenic or likely pathogenic variant. Eight (10%) participants had truncating *TTN* variants classified as variants of uncertain significance. Five (6%) participants had variants of unknown significance according to strict American College of Medical Genetics and Genomics criteria but classified as either pathogenic or likely pathogenic by other clinical laboratories. Pathogenic or likely pathogenic variants were found in 8 genes (all within tier 1 genes), 2 (20%) of which are not included in a standard commercially available dilated cardiomyopathy panel. Using our bioinformatics pipeline, there was an average of 0.74 variants of uncertain significance per case with  $\approx 0.75$  person-hours needed to interpret each of these variants.

**Conclusions**—Whole exome sequencing is an effective diagnostic tool for patients with dilated cardiomyopathy. With stringent classification using American College of Medical Genetics and Genomics criteria, the rate of detection of pathogenic variants is lower than previous reports. Efforts to improve adherence to these guidelines will be important to prevent erroneous misclassification of nonpathogenic variants in dilated cardiomyopathy genetic testing and inappropriate cascade screening. (*J Am Heart Assoc.* 2020;9:e013346. DOI: 10.1161/JAHA.119.013346.)

**Key Words:** cardiomyopathy • whole exome sequencing • clinical exome • next generation sequencing

Dilated cardiomyopathy (DCM) is a primary myocardial disorder characterized by left ventricular dilation and contractile dysfunction in the absence of abnormal loading conditions or coronary disease sufficient to cause global systolic dysfunction.<sup>1</sup> DCM is an important cause of death

and disability from heart failure and the disease course is determined by the underlying cause. DCM commonly has an underlying genetic basis, with causative variants identified in >60 genes of heterogeneous ontologies in both familial and apparently nonfamilial forms of disease.<sup>2–5</sup> Identification of a

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Accompanying Tables S1 through S5 are available at <https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.013346>

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## Clinical Perspective

### What Is New?

- Dilated cardiomyopathy commonly has an underlying genetic basis, with causative variants identified in >60 genes of heterogeneous ontologies.
- Using whole exome sequencing with stringent criteria for classifying genomic variants, pathogenic or likely pathogenic variants were identified in 12% of the study population.

### What Are the Clinical Implications?

- With stringent classification of variants using American College of Medical Genetics criteria, the rate of detection of pathogenic variants is lower than in previous reports.
- Despite the growing number of dilated cardiomyopathy-related genes, the incremental value of an extended gene set has not been convincingly demonstrated in the present study.
- Efforts to improve adherence to the American College of Medical Genetics criteria will be important to prevent erroneous misclassification of nonpathogenic variants in dilated cardiomyopathy genetic testing and inappropriate cascade screening.

genetic diagnosis is important not only to allow prognostication and possibly personalization of management but is essential also for family screening and to predict risk of recurrence. In contrast to conditions such as hypertrophic cardiomyopathy where up to 60% of hypertrophic cardiomyopathy patients carry mutations,<sup>6</sup> a genetic cause is less often identified in DCM, such that the role of routine genetic testing in DCM is less established and requires further study.

There is increasing evidence that clinical whole exome sequencing (WES) demonstrates a high diagnostic yield in achieving a molecular diagnosis of rare Mendelian disorders.<sup>7,8</sup> In contrast to panel testing, which involves testing a limited number of genes, WES provides genomic data on all protein coding sequences. In DCM it may have a role in reducing the number of unsolved cases without the additional costs of retesting as new genes are discovered. It is important to note, however, that broad analytical approaches in the evaluation of cardiomyopathies also have potential disadvantages. The higher number of genes tested allow an increase in diagnostic yield at the expense of longer turnaround time from sequencing and analytical processing with higher identification of variants of unknown significance, which increases clinical uncertainty.<sup>9</sup> Furthermore, WES-based analysis is associated with increased costs compared with targeted next generation sequencing approaches.<sup>9</sup>

Concerningly, up to 31% of all variants associated with DCM were subsequently identified in a recently published population exome browser, ExAC, questioning the pathogenic validity of many variants and/or genes previously thought to cause DCM.<sup>10</sup> As the estimated prevalence of DCM is 1:2500 in the general population, this genotype prevalence was up to 400-fold higher than the expected phenotypic prevalence of DCM.<sup>10</sup> Additionally, the reported diagnostic yield from previous research studies<sup>2,11,12</sup> comprising large multiplex families is expected to be higher than that can be achieved in a clinical setting. To date, the utility of WES as a first-line genetic test for patients with DCM in a contemporary “real-world” setting alongside routine clinical care has not been specifically established. Furthermore, the incremental value of an inclusive approach with the analysis of newer, lower-priority genes with indirect links to cardiac or skeletal myopathies is unknown.

We thus aimed to evaluate the rate of molecular diagnosis in a prospectively recruited cohort of patients with DCM using a WES approach.

## Subjects and Methods

### Study Design and Participants

Eligible participants were prospectively recruited from April 6, 2016 to September 24, 2017 from outpatient services of 4 tertiary cardiovascular centers in Melbourne Australia (Austin Health, Melbourne Health, Monash Health, and the Royal Children's Hospitals). The authors declare that all supporting data are available within the article and its online supplementary files.

The study cohort comprised patients for whom it was considered clinically appropriate to offer genetic testing in addition to their routine clinical care. We recruited patients with idiopathic DCM who were either (1) diagnosed under the age of 40 years, or (2) had a family history ( $\geq 2$  members per family including the proband) of DCM and/or early ( $< 35$  years) sudden unexplained death. Family history was extensively investigated by trained genetic counselors in centers with expertise in genetic cardiomyopathies and a  $\geq 3$ -generation pedigree was constructed with available information. Patients with known nongenetic causes or patients who had undergone prior genetic testing involving cardiomyopathy-related genes were excluded from the study.

Children (age  $< 18$  years) were only included after exclusion of metabolic, mitochondrial, and infective causes of cardiomyopathy. For patients suspected of having an infectious cause of cardiomyopathy (eg, viral myocarditis), viral cultures and/or titers were performed for the most common causes (eg, enterovirus, respiratory viruses, influenzae, adenovirus, parvovirus B19, Epstein-Barr virus, cytomegalovirus, and human herpes 6). Children with suspected mitochondrial

disorders presenting with distinct clinical syndromes including skeletal myopathies, neurological, and developmental symptoms were subjected to an extensive evaluation with involvement of a multidisciplinary team including geneticists and neurologists and by testing for mitochondrial mutations. Given the increased risks from endomyocardial biopsy, it was performed selectively, with the decision assessed individually for each patient based on the risk and benefit of the procedure. Endomyocardial biopsy was reserved for patients who required catheterization for another reason and/or those needing advanced heart failure management. A typical metabolic screen included assessment of serum amino acids, urine organic acids, acylcarnitine profile, lactate, pyruvate, electrolytes, glucose, and creatinine phosphokinase.

Potential participants were discussed by a panel of study investigators and the treating clinicians (JR, MW, IM, EL, SL, RW, TT, AT, DZ, JV, DH, and PJ) to determine eligibility before obtaining signed informed consent. All patients and/or parents provided written informed consent after genetic counseling regarding the test. To determine whether there were further affected members, family history was revisited in all patients during a mandated genetic counseling follow-up consultation.

The study was part of the Melbourne Genomics Health Alliance project (<http://www.melbournegenomics.org.au>).<sup>13</sup> The study received Human Research Ethics Committee approval (HREC/13/MH/326) from the Melbourne Health Human Research Committee. The investigation conforms with the principles outlined in the Declaration of Helsinki. Participants or parents provided written informed consent after genetic counseling.

## WES, Variant Detection, and Variant Filtering

We performed WES, variant detection, and filtering at the Victorian Clinical Genetics Services Laboratory as described previously.<sup>8,14</sup> The Nextera Rapid Capture Exome kit by Illumina (San Diego, CA) was used. Variants were characterized using the Melbourne Genomics Health Alliance shared bioinformatics pipeline, Cpipe<sup>14</sup> version 2.3. Cpipe is a clinically oriented pipeline built on Bpipe,<sup>15</sup> and includes the Burrows-Wheeler Aligner (BWA-0.7.5a-r405)<sup>16</sup> and the GenomeAnalysis ToolKit (GATK version 3.6)<sup>17</sup> using Haplo-typeCaller for variant calling.

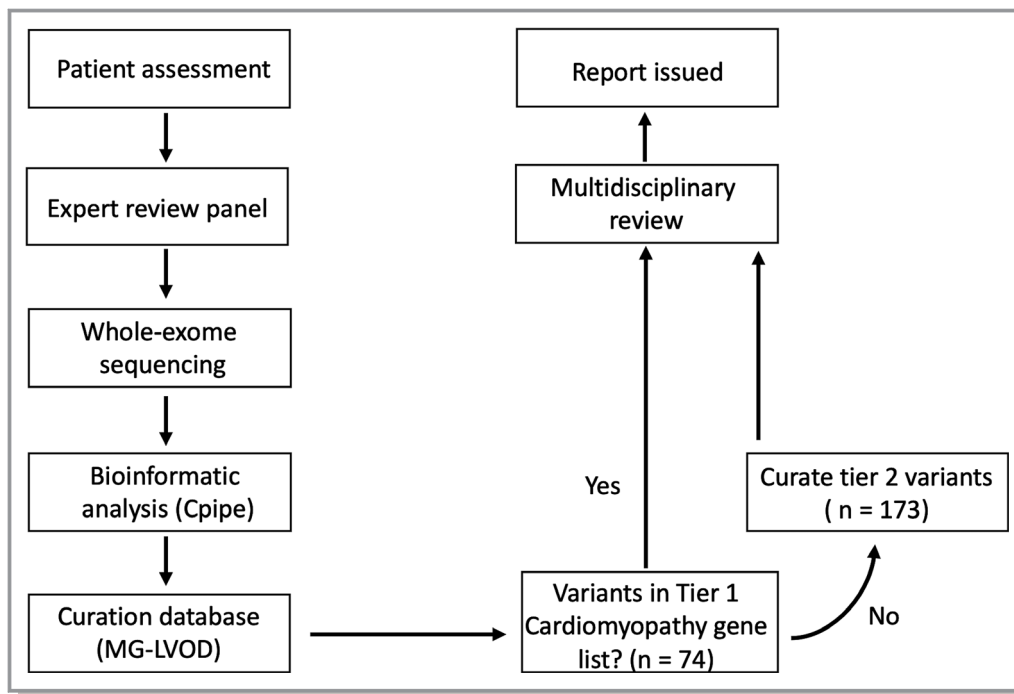
Variants were assessed using the Melbourne Genomics variant curation database, a modification of the Leiden Open Variation Database between April 6, 2016 and September 24, 2017.<sup>18</sup> Variants were prioritized based on a predetermined gene list for all participants and on predicted effect (Variant Prioritisation Index). The gene list was selected following extensive review of literature, online databases, and expert opinion. The list (Table S1) comprised high-priority or tier 1

clinical cardiomyopathy genes (n=74) and lower-priority or tier 2 genes that comprised general myopathy, mitochondrial genes, and other new or recently reported cardiomyopathy genes (n=173). If no pathogenic or likely pathogenic (P/LP) variants were identified following analysis of tier 1 genes, then variants in the tier 2 gene list were curated. Variants were rigorously classified based on principles outlined by the American College of Medical Genetics and Genomics (ACMG) standards for interpretation of sequence variants.<sup>19</sup> The in silico tools used were Polyphen2,<sup>20</sup> SIFT,<sup>21</sup> and Mutation Taster<sup>22</sup> and CADD.<sup>23</sup> Allele frequency was obtained from the Genome Aggregation Database (gnomADbrowser: <http://gnomad.broadinstitute.org>; accessed January 15, 2019). The disease database used was ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar>; accessed January 15, 2019). Variant classifications were reviewed in a multidisciplinary team meeting attended by clinical geneticists, cardiologists, genetic counselors, scientists, nurses, and bioinformaticians. Families with variant of uncertain significance (VUS) considered of probable clinical relevance were all invited for cosegregation testing. To avoid delays with initial release of results, our study protocol did not mandate that segregation testing be performed before initial variant adjudication. Variants were therefore adjudicated without results of segregation testing except in 1 individual with a suspected de novo variant, where segregation studies could be promptly performed. The processes for participant recruitment and data analysis are summarized (Figure 1).

Exome sequencing results emanating from this laboratory have previously been validated across a wide spectrum of clinical disorders and are accredited by the Australian National Association of Testing Authorities (NATA) for routine clinical use, without any mandate of orthogonal validation of results.<sup>8,24–27</sup>

Challenges exist in assigning pathogenicity to rare truncating *Titin* (*TTN*) variants given the prevalence in the general population. On the basis of a recent, comprehensive study of the *TTN* gene and its transcripts, nonsense, frameshift, and canonical splice site *TTN* variants, particularly those that truncate both principal isoforms of *TTN* and/or reside towards the C terminus, were considered to have a higher likelihood of pathology.<sup>28</sup> In contrast, truncations that occur in novex-specific exons or other infrequently used *TTN* exons were considered less likely to be deleterious.<sup>28</sup>

Other approaches including single-nucleotide polymorphism microarray analysis and/or multiplex ligation-dependent probe amplification were performed as needed either before recruitment or following review by the multidisciplinary team (N=5). Dedicated Sanger sequencing was used when thought to be clinically appropriate to genotype further family members to observe whether the variant cosegregated with disease. Formal quantification of cosegregation was not made.



**Figure 1.** Processes of patient enrollment and variant curation. LP/P indicates likely pathogenic or pathogenic; MG-LOVD, Melbourne Genomics-Leiden Open Variation Database.

## Statistical Analysis

Statistical analysis was performed using STATA, version 15.1 (Statacorp., College Station, TX). Normally distributed continuous variables are expressed as mean $\pm$ SD and non-normally distributed data are expressed as the median and interquartile range. Student *t* test or the Mann Whitney *U* test (for non-normally distributed data) was used to assess differences in continuous variables. Categorical variables are expressed as counts and percentages and compared using Fisher exact or  $\chi^2$  tests.

The main outcome of the study was the proportion of patients with P/LP variants. Adverse clinical outcomes such as heart failure hospitalization, heart transplantation, and death were collected by experienced investigators via medical records review and by contacting each patient and/or the nominated general practitioner for additional information.

## Results

Of 90 eligible patients evaluated, we enrolled 83 unrelated probands with DCM. The 7 patients excluded had a probable nongenetic cause for their condition. The clinical characteristics of the probands are presented in Table 1. The majority of participants were adults ( $\geq 18$  years,  $n=72$ , 87%). The overall cohort comprised 57% males ( $n=47$ ) with a mean $\pm$ SD age of  $36\pm 19$  years. Most had a family history of cardiomyopathy and/or early sudden unexplained death ( $n=60$ , 72%). Forty-eight of 83 probands (58%) had a family history of only

cardiomyopathy and 6 of 83 (7%) had a family history of only sudden death. The remaining participants ( $n=23$ , 28%) did not have a known family history of cardiomyopathy or sudden death affecting first- and second-degree relatives and met study inclusion criteria on the basis of a diagnosis of cardiomyopathy under the age of 40 years. Most families with familial DCM/sudden cardiac death had 1 additional family member (33 of 60, 55%) with DCM/sudden cardiac death, with 15 (25%) having 2 and 12 (20%), respectively, having  $>2$ . There was a high incidence of atrial ( $n=18$ , 22%) and ventricular arrhythmias ( $n=10$ , 12%). With regard to pharmacological therapy, of the entire cohort, 72 (87%) were on angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, 69 (83%) on  $\beta$ -blockers, and 38 (46%) on mineralocorticoid receptor antagonists.

## Genetic Diagnosis

We generated high-coverage WES data with 97% of tier 1 and tier 2 genes covered by  $>20$  reads. Genes without 100% of coverage of  $>20$  reads are listed in Table S2. Of the 83 probands, 10 (12.0%) had a pathogenic ( $n=1$ ) or likely pathogenic ( $n=9$ ) variants (Table 2 and Figure 2).<sup>29–33</sup> There was a nominally higher rate of genetic diagnosis in pediatric compared with adult cases (18% versus 11%). Among the pediatric probands ( $n=11$ ), there was no significant difference in the rate of P/LP variants in those with (1 of 5, 20%) or without a family history of DCM or sudden cardiac death (1 of

**Table 1.** Proband Characteristics

	Whole Cohort (n=83)	Isolated (n=23)	Familial (n=60)	P Value
Age at diagnosis (y)	37±19	25±14	42±18	<0.001*
Male	47 (57%)	11 (48%)	36 (60%)	0.317
LVEF at time of diagnosis, %	31±13	30±14	31±13	0.868
Number of first-degree family relatives	4 (3–6)	4 (3–5)	5 (4–6)	0.131
Number of second- or higher-degree relatives	15 (10–20)	15 (10–26)	15 (11–20)	0.983
Comorbidities/therapy at time of recruitment				
Prior heart failure admission	51 (61%)	14 (61%)	37 (62%)	0.947
Atrial fibrillation	18 (22%)	5 (22%)	13 (22%)	0.977
Ventricular tachycardia	10 (12%)	5 (22%)	5 (8%)	0.106
Diabetes mellitus	10 (12%)	3 (13%)	7 (12%)	0.883
β-Blocker	69 (83%)	18 (78%)	51 (85%)	0.269
ACEi/ARB	72 (87%)	19 (83%)	53 (88%)	0.257
Mineralocorticoid receptor antagonists	38 (46%)	9 (39%)	29 (48%)	0.377
Implantable cardiac defibrillator	22 (27%)	4 (17%)	18 (30%)	0.244
Cardiac resynchronization therapy	14 (17%)	5 (22%)	9 (15%)	0.463

Values are mean±SD or median (interquartile range) or n (%). ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; LVEF, left ventricular ejection fraction.

\*Denotes  $p < 0.05$ .

6, 17%). Of the adult cases (n =72), there was a nominally higher number of patients with P/LP variants in those with a family history (7 of 55, 13%) compared with isolated cases (1 of 17, 6%), though this did not reach statistical significance ( $P=0.433$ ). Each ACMG rule activated to classify P/LP variants as well as rare VUS is detailed in Tables S3 and S4. Eight of 83 patients (9.6%) had a truncating TTN VUS (Table 3). Taken together, P/LP variants were found in 8 genes (all within tier 1 genes), 2 (20%) of which are not included in a standard commercially available DCM panel (Table S5). However, as the 2 patients involved had arrhythmogenic phenotypes, it is likely that they would have received a comprehensive cardiac panel comprising these 2 genes (*DSP* and *RYR2*).

Of note, 59 variants of uncertain significance were identified in tier 1 genes and 3 variants of uncertain significance were identified in tier 2 genes. On average there were 0.74 (61/83) variants of uncertain significance per case. Time to curate each variant was approximated to be 0.75 person-hours. Five of 83 (6%) participants had variants of unknown significance according to strict ACMG criteria but classified as either P/LP by other clinical laboratories. One individual was found to have 1p36.32 deletion on chromosome SNP microarray testing.

### Impact of Genetic Diagnosis on Patient Management

Of the 10 patients with a P/LP result, clinical management was altered following genetic testing in 1 individual (10%). One

patient (Subject 5, Table 2) underwent implantable cardiac defibrillator implantation for primary prevention of sudden cardiac death following identification of a *LMNA* variant.

### Clinical Outcomes

Median follow-up time was 501 days. Over the follow-up period, there was no significant difference in the incidence of heart failure–related admissions (2 of 10 [20%] versus 6 of 73 [8%],  $P=0.236$ ), treatment with left ventricular assist device, or cardiac transplantation (0 [0%] versus 3 of 73 [4%],  $P=0.514$ ) or death (1 of 10 [10%] versus 2 of 73 [3%],  $P=0.249$ ) between those with or without P/LP variants.

### Discussion

We performed singleton WES as a first-line genomic test in a prospectively enrolled cohort of patients with DCM enrolled from routine clinical practice. The major finding of the present study was that WES identified a likely P/LP variant in 12% of patients with DCM in a “real-world” clinical setting. A further 10% of patients had truncating *TTN* variants classified as VUS. No variants in the tier 2 (extended gene set data) met clinical criteria for pathogenicity. Concerningly, 5 (6%) participants had variants of unknown significance according to strict ACMG criteria, but such variants were classified as either P/LP by other clinical laboratories.



**Table 2.** Characteristics of Patients With Likely Pathogenic and Pathogenic Variants

Subject, Sex	Age(y)	Phenotype	Gene (Ref Seq Access number): Variant	Predicted Canonical Protein Isoform	Variant Co-Segregation*	In Silico Prediction Tools	Previously Reported Pathogenic Variant	Functional Data
<b>Pathogenic</b>								
2, F	65	LV dysfunction diagnosed following VF arrest. ECG: Left anterior fascicular block, septal Q waves.	NM_001001432.2(TNNT2): c.517C>T	NP_001001432.1(TNNT2): p.(Arg173Trp)	Not performed	Consistently pathogenic	Yes <sup>30,32</sup>	Yes <sup>33</sup>
<b>Likely pathogenic</b>								
4, M	53	Moderate to severe LV dysfunction; ECG: Leftward axis, mild QT prolongation.	NM_003319.4(TTN): c.69855dupA	NP_001254479.1(TTN): p.(Glu32351Argfs*6)	Not performed	Consistently pathogenic	No, other truncating variants in region reported as pathogenic	No
6, F	29	Severe biventricular dysfunction. ECG: rightward axis, deep widespread T wave inversion and QT prolongation.	NM_003319.4(TTN): c.21991G>T	NP_001254479.1(TTN): p.(Glu16396*)	Not performed	Consistently pathogenic	No. Downstream variants reported as pathogenic (ClinVar)	No
8, F	52	Moderate LV dysfunction. ECG: left anterior fascicular block.	NM_003319.4(TTN): c.44430_44449del	NP_001254479.1(TTN): p.(Val23876Argfs*16)	1/0/0	Consistently pathogenic	No	No
9, F	49	Familial dilated cardiomyopathy, ECG: atrial fibrillation	NM_133378.4(TTN): c.10361-3042T>A	NP_001254479.1(TTN): p.(Leu3944*)	1/0/0	Consistently pathogenic	No	No
1, F	23	Mild LV dysfunction; Cardiac arrest because of ventricular fibrillation (VF). Family history of SCD. Recurrent ventricular tachycardia. ECG: low voltage R waves.	NM_004415.2(DSP): c.2638dupG	NP_004406.2(DSP): p.(Asp880Glyfs*14)	1/0/0	Consistently pathogenic	No. Downstream variants reported as pathogenic (ClinVar)	No
3, M	12	Biventricular dysfunction diagnosed in the setting of multifocal atrial tachycardia. Multiple foci in the left and right atria on EPS.	NM_001035.2(RYR2): c.14570T>A <sup>†</sup>	NP_001026.2(RYR2): p.(Ile4857Asn)	0/0/0	Consistently pathogenic	No	No
5, M	31	Recurrent atrial and ventricular arrhythmias. Biventricular dysfunction. Family history of SCD. ECG: RBBB.	NM_170707.3(LMNA): c.1608+1G>A	NP_733821.1(LMNA): p.?	1/0/0	Predicted to cause aberrant splicing	Yes <sup>29</sup>	No
7, F	57	Moderate LV dysfunction. ECG: left anterior fascicular block, widespread T wave inversion.	NM_004281.3(BAG3): c.108G>A	NP_004272.2(BAG3): p.(Trp36*)	Not performed	Consistently pathogenic	No. Downstream variants reported as pathogenic (ClinVar)	No

Continued

Table 2. Continued

Subject, Sex	Age(y)	Phenotype	Gene (Ref Seq Access number): Variant	Predicted Canonical Protein Isoform	Variant Co-Segregation*	In Silico Prediction Tools	Previously Reported Pathogenic Variant	Functional Data
10, M	5	LV noncompaction with mild LV dilatation and normal LV systolic function.	NM_005159.4(ACTC1):c.998C>T	NP_005150.1(ACTC1):p.(Ala333Val)	1/0/0	Consistently deleterious	Yes <sup>31</sup>	No

No allele frequencies were available as all were absent from GnomAD. References are shown as superscripts. EPS indicates electrophysiology studies; LV, left ventricular; RB8B, right bundle branch block; SCD, sudden cardiac death; VF, ventricular fibrillation.  
\*Data presented as A/B/C where A=number of affected family members (excluding the proband) who carry the variant, B=number of affected members who do not carry the variant, C=number of unaffected members who carry the variant.  
<sup>†</sup>Subsequent testing of parental samples indicated that this variant is likely because of a de novo event.

Our data indicate that with stringent classification according to contemporary ACMG guidelines, the rate of detection of pathogenic variants in the present study performed in a clinical setting is lower than previously reported.<sup>2,11</sup> Indeed, concerning, several lines of evidence suggest previous overreporting of many rare protein-altering variants as pathogenic, with such variants subsequently being detected in the general population, raising doubts with regard to the reliability of previously released genetic results.<sup>34–38</sup> Assigning pathogenicity of rare genetic variants is particularly challenging with the enormous *TTN* gene that encodes titin, the largest human protein spanning the entire sarcomere of heart and skeletal muscle tissue.<sup>35</sup> Truncating *TTN* variants have been shown to occur in up to 3% of apparently healthy individuals.<sup>35,39</sup> It is therefore imperative to distinguish truncating *TTN* variants that cause disease from those that do not. We note that other laboratories<sup>11</sup> classify many such variants as pathogenic and hence comparatively this alone would account for the lower rate of pathogenic diagnoses in the present study.

Detailed exon use data from the human myocardium demonstrate that I-band titin contained a high proportion of symmetrical exons with a very low “percentage spliced-in” score in expressed exons.<sup>28</sup> Variants that truncate exons consistently expressed in both of the dominant N2BA and N2B isoforms have an increased likelihood of pathogenicity with truncations involving the C-terminal portion of the protein, encoding the A-band titin, being the predominant class of pathogenic variants.<sup>28</sup> Use of the aforementioned criteria help resolve the majority of truncating *TTN* variants, but strikingly truncating *TTN* variants that fit the criteria for pathogenicity can still be seen in ≈1% of apparently normal individuals.<sup>40</sup> It is nonetheless important to emphasize that DCM may develop at any age and therefore population databases have incomplete ascertainment of cardiovascular outcomes. It is therefore possible that although the high frequency of some of these *TTN* variants preclude them from being the driving causative factors, lower-frequency variants may be making a significant contribution to phenotype. Indeed, in a detailed analysis of population data involving >71 000 individuals, Haggerty et al<sup>41</sup> recently reported the association between high percentage spliced-in truncating *TTN* variants and preclinical cardiac abnormalities even in those without a DCM diagnosis.

At the present time, we have chosen to conservatively classify all apart from the most likely to be pathogenic truncating *TTN* variants as VUS (Table 3) until further evidence is gathered. Of the 8 variants in this cohort listed in Table 3, 4 do not have a high percentage spliced-in (subjects 17, 19, 20, and 21) and 2 (subjects 14 and 16) do not encode the A-band, rendering the pathogenicity of these variants uncertain. The remaining 2 rare variants have a higher likelihood of pathogenicity (subjects 15 and 18) as they

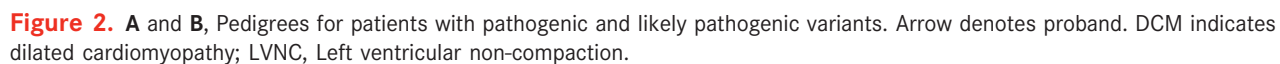




Table 3. TTN Variants of Uncertain Significance

Subject, Sex	Exon Number	Canonical Isoform Nomenclature	Predicted Canonical Protein Isoform	Affecting N2BA & N2B	Protein Region	In Silico Tools	PSI	Population Database
14, M	29	NM_001267550.1:c.6825del	NP_001254479.1(TTN);p.(Asp2275Gluufs*2)	Both	I-band	Pathogenic	1.0	Absent
15, M	154	NM_001267550.1:c.80174_80202dup	NP_001254479.1(TTN);p.(Ser26735Glnfs*9)	Both	A-band	Pathogenic	1.0	Absent
16, M	Splice-site (Intron 54)	NM_001267550.1:c.41609-2A>G	NP_001254479.1(TTN);p.?	Both	N/A	Aberrant splicing	1.0	Absent
17, M	94	NM_001267550.1:c.50170C>T	NP_001254479.1(TTN);p.(Arg16724*)	Both	A-band	Pathogenic	0.60	Absent
18, F	29	NM_001267550.1:c.60961_60962dupAA	NP_001254479.1(TTN);p.(Asn20321Lysfs*9)	Both	A-band	Pathogenic	1.0	Absent
19, M	153	NM_001267550.1:c.69422_69426delinsAAAGGACCC	NP_001254479.1(TTN);p.(Gly23141Gluufs*38)	Both	A-band	Pathogenic	0.48	0.04%
20, F	186	NM_001267550.1:c.101107C>T	NP_001254479.1(TTN);p.(Arg33703*)	Both	M-band	Pathogenic	0.07	Absent
21, M	9	NM_001267550.1:c.50296C>T	NP_001254479.1(TTN);p.(Arg16766*)	Both	A-band	Pathogenic	0.64	<0.00041%

N/A indicates not applicable; PSI, percentage spliced in.

involve the A-band and have high percentage spliced-in scores and hence familial cosegregation studies are planned to further establish their pathogenicity.

Other reasons for a lower yield of identifying P/LP variants in this study include the careful interpretation of ACMG criteria in the interpretation of loss of function variants. This criterion is the only one assigned PVS1 (very strong evidence for pathogenicity) and hence extra caution was used in the present analysis to prevent overestimation of variant impact and subsequent classification. Use of recent recommendations to interpret loss of function variants from ClinGen’s Sequence Variant Interpretation working group will help promote consistent interpretation of loss of function variants among laboratories.<sup>42</sup> In DCM, further refinement of these criteria will be required by integrating gene-level and variant-level characteristics.<sup>34</sup>

Interestingly, there was no significant difference with regard to family history or age of onset between those with and without a molecular diagnosis. These findings highlight that lack of family history of cardiomyopathy and later age of disease onset do not exclude a genetic cause, especially as certain environmental factors may interact to induce pathology at a later stage in life.<sup>39</sup>

Despite the growing number of DCM-related genes, the incremental value of an extended gene set, beyond which would be included in a comprehensive cardiac panel, has not been convincingly demonstrated in the present study. Not unsurprisingly, no variants in the extended (tier 2) gene set met clinical criteria for pathogenicity using stringent criteria of the ACMG. By definition, genes included in the extended data set included those that were of lower priority and/or newly reported, and thus one of the challenges in evaluating variants in the extended gene set is paucity of genetic and functional effects for DCM association. These findings are in keeping with a recent study of whole genome sequencing in DCM that included 406 cardiac-enriched genes but found that no variants in the extended gene set met clinical criteria.<sup>12</sup> Importantly, however, as evidence of disease associations build in the extended gene list, data from exome sequencing, much of which cannot be obtained from panel sequencing, can be reanalyzed in the future.

Our results indicate that with the use of our Melbourne Genomics Health Alliance shared bioinformatics pipeline, WES with a targeted analysis was time efficient with an average of 0.74 variants of uncertain significance per case and 0.75 person-hours needed to interpret each of these variants. The manpower costs of expert clinical reporting would therefore be expected to be equivalent for panel testing and WES.

The strengths of this study include its demonstration of the utility of singleton, genomic testing in “real-life” care of patients with DCM. Only ≈70% of patients had a family history of cardiomyopathy, and in fact most of the probands

only had 1 other first- or second-degree affected relative and therefore the likelihood of finding pathogenic variants are unsurprisingly substantially lower than in large multiplex families typically observed in research settings. Some important limitations should be acknowledged. Although family history was extensively investigated and every effort was made to invite first- and second-degree relatives of probands for further cardiovascular evaluation, this was not comprehensively adhered to. Thus, the presence of a family history of DCM may have been underrecognized in some probands. WES has limited sensitivity for the detection of insertion/deletion variants over 10 bp in length, copy number variation, translocations, chromosomal rearrangements, and repeat expansions. Other limitations of exome sequencing include the potential of missing variants because of variability in breadth of coverage involving certain genes such as *LMNA* and low mappability of certain sarcomeric genes. Comprehensive cardiac gene panels with associated higher coverage and lower costs may have achieved similar findings. However, WES has added the advantage for future re-analysis of data as new DCM-related genes are identified.

In conclusion, WES with targeted gene analysis is an effective diagnostic tool for patients with DCM in a clinical setting. The data from this study indicate that with stringent classification, according to current evidence, the rate of detection of pathogenic variants in DCM is lower than previously reported. Ongoing efforts to improve adherence to these guidelines may prevent erroneous misclassification of nonpathogenic variants in DCM genetic testing and cascade screening.

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## Disclosures

None.

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