ARTICLE

Diagnostic and prognostic relevance of using large gene panels in the genetic testing of patients with dilated cardiomyopathy

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It was previously suggested that increasing the number of genes on diagnostic gene panels could increase the genetic yield in patient with dilated cardiomyopathy (DCM). We explored the diagnostic and prognostic relevance of testing DCM patients with an expanded gene panel. The current study included 225 consecutive DCM patients who had no genetic diagnosis after a 48-gene cardiomyopathy-panel. These were then evaluated using an expanded gene panel of 299 cardiac-associated genes. A likely pathogenic/pathogenic (P/LP) variant was detected in 13 patients. Five variants were reclassifications of variants found in genes which were already detected using the 48 gene panel. Only one of the other eight variants could explain the phenotype of the patient (*KCNJ2*). The panel detected 186 VUSs in 127 patients (of which 6 also had a P/LP variant). The presence of a VUS was significantly associated with the combined end-point of mortality, heart failure hospitalization, heart transplantation or life-threatening arrhythmias(HR, 2.04 [95% CI, 1.15 to 3.65]; $p = 0.02$). The association of a VUS with prognosis remained when we only included VUSs in robust DCM-associated genes (high suspicious VUSs), but disappeared when we only included VUSs in non-robust DCM-associated genes (low suspicious VUSs), highlighting the importance of weighing of VUSs. Overall, the use of large gene panels for genetic testing in DCM does not increase the diagnostic yield, although a VUS in a robust DCM-associated gene is associated with an adverse prognosis. Altogether, current diagnostic gene panels should be limited to the robust DCM-associated genes.

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INTRODUCTION

Dilated cardiomyopathy (DCM) is defined as the presence of left ventricular or biventricular dilatation and systolic dysfunction in the absence of abnormal loading conditions (hypertension, valvular disease) or coronary artery disease sufficient to cause global dysfunction [1]. Genetic testing has become a first-tier diagnostic test for every patient with DCM according to the latest guidelines [2–4]. A pathogenic or likely pathogenic (P/LP) gene variant is found in ~20% of DCM patients [5]. The genetic landscape of DCM has greatly expanded, and over 60 genes are currently associated with DCM in the Human Gene Mutation Database (HGMD). Whether variants in these genes are of pathogenic relevance for monogenetic DCM has been disputed [6–8], and the number of robust DCM-associated genes may be as low as 15–20. This debate is reflected in gene panels used in genetic testing for DCM decreasing in number of genes [8]. On the other hand, a significant group of DCM patients with a strong family history of DCM remains without a genetic etiology [5], and a previous systematic literature search suggested to increase the number of genes in diagnostic gene panels to increase the genetic yield in DCM patients [9]. However, the suggestion of genetic testing with large gene panels in a clinical setting has not been systematically tested yet, therefore we do not know if large gene panels effectively increase the genetic yield. It has been proposed that complex polygenic and multifactorial models of DCM could explain the undetected genetic inheritance in these families [10, 11]. Before advancing to the era of polygenic risk models, we evaluated the diagnostic yield of a large gene panel using exome sequencing in a cohort of DCM patients. By composing a large panel of genes associated with cardiac disease, we explored whether a large panel can increase the genetic yield in patients with DCM compared to a small panel of DCM-associated genes, and determined the clinical relevance of detected variants.

METHODS

Study population

The study population consisted of 225 unrelated DCM probands who were all included in the Maastricht Cardiomyopathy Registry (mCMP-registry) which prospectively included patients from the out-patient clinic between 2004 and 2022 [12]. All individuals older than 16 years of age who are

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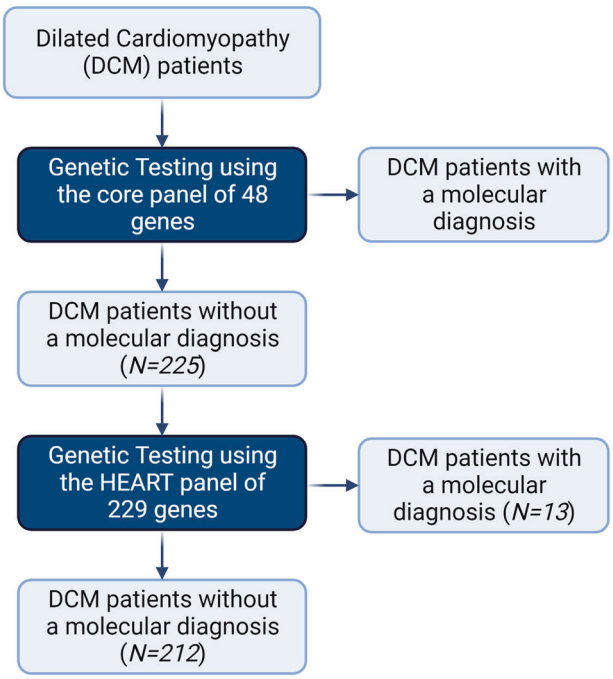

Fig. 1 Flow chart of genetic testing in DCM patients. A total of 225 patients did not receive a molecular diagnosis after genetic testing using the core panel of 48 genes. Patients who did received a molecular diagnosis (finding of a P/LP variant) will not receive further genetic testing and were excluded from the analysis. The 225 DCM patients without a molecular diagnosis were further tested using a broad HEART panel consisting of 229 genes. Only 13 patients received a molecular diagnosis after genetic testing using the HEART panel.

referred to the cardiology department of the Maastricht University Medical Centre (MUMC+, Maastricht, The Netherlands) for heart failure-like symptoms or screening for cardiomyopathies are eligible for inclusion. The DCM diagnosis was defined according to the World Health Organization criteria and the latest ESC proposal. Enrolled patients presented with a left ventricular ejection fraction (LVEF) below 50% at baseline echocardiographic evaluation in the absence of any of the following conditions: obstruction >50% of a major coronary artery branch [at coronary angiography (CAG)], pericardial diseases, primary valvular disease, cor pulmonale, and active myocarditis. All patients underwent a physical examination, blood sampling, 12-lead ECG, 24-hour Holter monitoring, genetic testing, a complete echocardiographic and Doppler evaluation, and coronary angiography at baseline.

For the current study patients with DCM were selected if they (1) had no genetic diagnosis after testing using our core panel of 48 cardiomyopathy-associated genes [5] and (2) underwent subsequent genetic testing using an expanded 299 gene HEART panel (Fig. 1). The study was performed according to the declaration of Helsinki and was approved by the institutional Medical Ethics Committee. All patients gave written informed consent.

Genetic analysis

DCM patients were referred for genetic counselling and DNA testing to the Clinical Genetics department of the MUMC+. We make distinction and refer to three different gene panels:

1. Robust DCM-associated gene panel – containing 14 genes (robust panel): *TTN, DSP, MYH7, LMNA, BAG3, TNNT2, TNNC1, PLN, ACTC1, NEXN, TPM1, VCL, RBM20*, and *FLNC*.
2. DCM core panel – containing 48 genes (core panel): genes from the robust panel + *ACTN2, ANKRD1, CALR3, CAV3, CRYAB, CSRP3, CTNNA3, DES, DSC2, DSG2, EMD, FHL1, GLA, JPH2, JUP, LAMA4, LAMP2, LDB3, MIB1, MYBPC3, MYH6, MYL2, MYL3, MYOZ2, MYPN, PKP2, PRDM16, PRKAG2, SCN5A, TAZ, TCAP, TMEM43, TNNI3*, and *TPM1*.
3. The extended HEART panel – containing 299 genes (HEART panel): The latest version of the HEART panel can be found on https://order.radboudumc.nl/en/products/wes-heart-disorders1. Current version includes 299 genes that are associated with cardiac disease (Table S1). Genes are included in the panel based on literature describing the gene in association with human cardiac disease and expert consensus. A copy number variation (CNV) analysis of the included genes is included in the panel analysis.

Patients underwent genetic testing using a 299 gene HEART panel when previous genetic testing using our DCM core panel was negative for (likely) pathogenic variants. All variants were classified according to the ACMG guidelines [13, 14]. A family history of cardiac-related disease and sudden cardiac death was obtained by a 3-generation pedigree analysis at the initial visit of the patient. Familial inheritance was defined as recommended by the ESC [1]: (i) two or more individuals (first or second-degree relatives) have DCM fulfilling diagnostic criteria for 'definite' disease OR (ii) in the presence of an index patient fulfilling diagnostic criteria for DCM and a first-degree relative with autopsy-proven DCM and sudden death at <50 years of age.

Follow-up

The median follow-up time was 5.1 years (interquartile range 4.1 to 7.9 years). Information about the occurrence of adverse events at follow-up was retrieved from the hospital medical records, the Dutch Personal Records Database and/or telephone contact with the patient or their general practitioners. We collected information regarding four different adverse events: (1) death due to cardiac disease, (2) heart transplantation or LVAD implantation, (3) heart failure that required a non-elective hospitalization despite optimal heart failure therapy according to the ESC/ACC/AHA guidelines, and (4) life-threatening arrhythmias (LTA) defined as non-fatal ventricular fibrillation (with or without ICD-shock), and/or sustained ventricular tachycardia with appropriate ICD shock. The prognosis was defined as a combination of end-points and was specified as the occurrence of at least one of the above-mentioned adverse events.

Statistical analysis

Kaplan–Meier survival curves were estimated and differences between groups were assessed by the log-rank test, using time of DCM diagnosis as time zero. Cox proportional hazards regression analysis was performed to assess the association between the number and presence of VUSs with event-free survival. Calculations were done using R environment version 3.5 (R Foundation, Vienna, Austria).

RESULTS

Study population

In total, 225 patients with DCM underwent genetic testing using the HEART panel. All patients were previously tested using our core panel of 48 genes. The mean age of DCM diagnosis was 56 years (SD 12.22, range 20–80). 31 percent (69/225) of probands reported a family history of DCM; all 69 had at least one first-degree relative who had a diagnosis of DCM confirmed through retrieving medical files. 61 percent (138/225) of the DCM probands were male. The median ejection fraction was 39 percent (interquartile range 27–47), with a mean indexed left ventricular end-diastolic diameter of 29 mm/m^2 (SD 3.4, range 21–41). Seventeen percent of probands (38/225) had atrial fibrillation.

Genetic yield of the HEART panel

A P/LP variant was reported in 5.8% (13/225) of patients (Table 1), and 3 of the 13 patients had a familial form of DCM. Five of the thirteen variants would also have been reported upon testing with the 48 gene core panel (Fig. 2). Only one of the other eight variants could explain the DCM phenotype.

Four variants were already identified by the core panel, but were classified as a VUS at the time of reporting. A pathogenic variant in Filamin C (*FLNC*) was reported (c.6864_6867dup; p.(Val2290Argfs*23)) in one patient. *FLNC* is currently included in our core panel since 2018, but *FLNC* variants were not described as causative for cardiomyopathy at the moment of initial genetic

Table 1. All detected pathogenic or likely pathogenic variants in patients with dilated cardiomyopathy after genetic testing using a panel of 299 genes.

Gene	Refseq	Nucleotide consequence	Amino Acid consequence	Class	ACMG criteria†	Associated phenotype	Comment
TTN*	NM_001267550.2	c.13100del	p.(Lys4367Argfs*27)	LP	PP1_strong, PM2	MIM:604145; Cardiomyopathy, dilated type 1 G	Initially classified as VUS
DSC2*	NM_024422.6	c.21dup	p.(Gly8Argfs*23)	LP	PVS1, PM2	MIM:610476; Arrhythmogenic right ventricular dysplasia type 11	Initially classified as VUS
LMNA*	NM_170707.4	c.1517 A > C	p.(His506Pro)	LP	PS3, PM2, PP3	MIM:115200; Cardiomyopathy, dilated type 1 A	Initially classified as VUS
LMNA*	NM_170707.4	c.236 C > A	p.(Ala79Asp)	LP	PS3, PM2	MIM:115200; Cardiomyopathy, dilated type 1 A	Initially classified as VUS
FLNC*	NM_001458.5	c.6864_6867dup	p.(Val2290Argfs*23)	P	PVS1, PM2, PS4_supporting	MIM:617047; Cardiomyopathy familial	Gene was not in the initial genetic test
HFE	NM_000410.4	c.845 G > A and c.187 C > G	p.(Cys282Tyr) and p.(His63Asp)	P	PS3, PS4, PP1, PP3	MIM:235200; Hemochromatosis	Not associated with DCM
HFE	NM_000410.4	c.845 G > A and c.187 C > G	p.(Cys282Tyr) and p.(His63Asp)	P	PS3, PS4, PP1, PP3	MIM:235200; Hemochromatosis	Not associated with DCM
HFE	NM_000410.4	c.845 G > A and c.187 C > G	p.(Cys282Tyr) and p.(His63Asp)	P	PS3, PS4, PP1, PP3	MIM:235200; Hemochromatosis	Not associated with DCM
NODAL	NM_018055.5	c.323 C > G	p.(Ser108*)	LP	PVS1, PM2	MIM:270100; Heterotaxy, visceral type 5	Not associated with DCM
CACNA1C	NM_000719.7	c.1097 C > T	p.(Thr366Met)	LP	PM1, PM2, PP3, PP5	MIM611875; Brugada Syndrome type 3 and MIM:618447; Long QT Syndrome type 8	Not associated with DCM
SCN3B	NM_018400.4	c.516 G > A	p.(Trp172*)	LP	PVS1, PM2	MIM:613120; Brugada Syndrome type 7	Not associated with DCM
KCNQ1	NM_000218.3	c.899 C > G	p.(Ala300Gly)	LP	PM1, PM2, PM5, PP3	MIM:192500; Long QT Syndrome type 1	Not associated with DCM
KCNJ2	NM_000891.3	c.224 C > G	p.(Thr75Arg)	P	PS3, PM1, PM2, PP1, PP3	MIM:170390; Andersen-Tawil Syndrome	-

LP likely pathogenic, *P* pathogenic, *VUS* variant of unknown significance.
An asterisk indicates that the gene is also included in the core panel of 48 genes.
† All variants are classified according to the applicable ACMG criteria [13, 14].

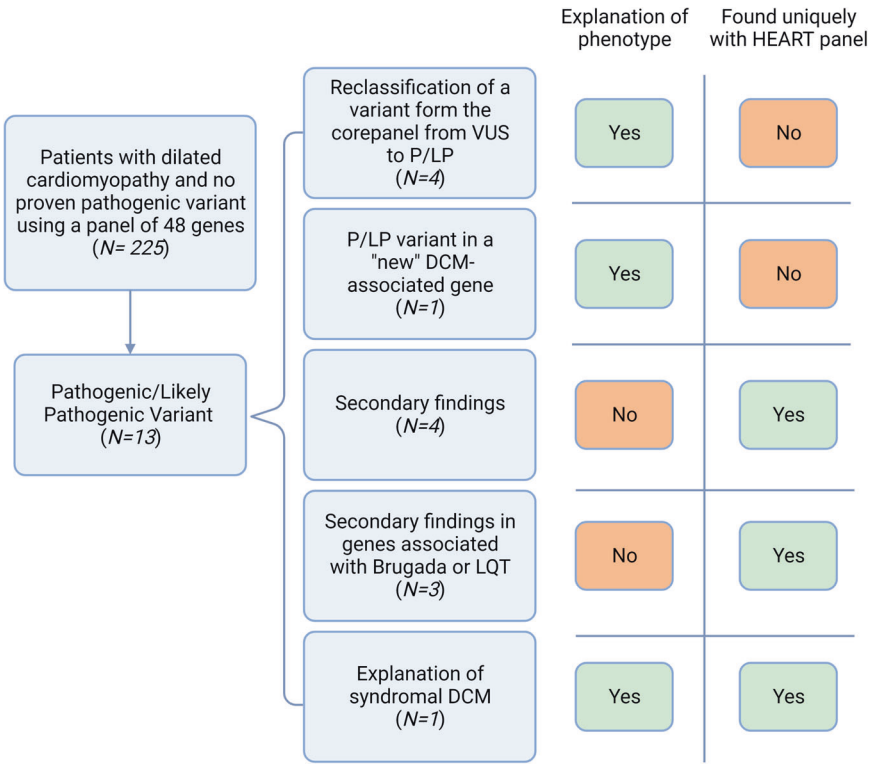

Fig. 2 Genetic yield of the HEART panel. A pathogenic or likely pathogenic variant in one of the 299 tested genes was detected in 13 of the 225 patients with dilated cardiomyopathy (DCM). Five variants would also have been detected upon testing with the current smaller panel of 48 genes, seven variants are stated as secondary findings as these do not explain the phenotype of the patient, and one variant was a monogenic cause of a syndromal form of DCM (Andersen-Tawil syndrome).

testing in this patient in 2015. Three patients were compound heterozygous for the *HFE* variants p.(Cys282Tyr) and p.(His63Asp), but did not have signs of iron overload. The *HFE* variants were therefore not explanatory for the DCM phenotype. Also, diagnosis of the most common form of *HFE*-related hemochromatosis (homozygous Cys282Tyr) is excluded [15]. One patient had a likely pathogenic variant in *NODAL*, which is a gene encoding a TGF-β subfamily ligand involved in the mesoderm and endoderm development. Heterozygous *NODAL* variants have been described in patients with congenital heart defects such as transposition of the great vessels and isolated septal defects, but not with DCM. Finally, four patients had a P/LP variant in a gene associated with hereditary rhythm disorders such as Brugada and long QT syndrome (variants in *CACNA1C, KCNJ2, KCNQ1*, and *SCN1B*, respectively; Table 1). These patients were evaluated for a primary rhythm disorder. In total 186 VUSs were identified in 127 patients (56.4%) using the HEART panel, of which 88 VUSs were already reported by the core panel in 69 patients (30.6%; Figure S1, Table S2). There were 58 patients for whom a VUS was reported after genetic testing using the HEART panel in a gene outside the core panel (25.8%). No pathogenic CNV was detected. All reported variants are shown in Table S3, and per patient in Figure S2 and Table S6.

Association between genetic variants and the patient phenotype

Four patients had a LP variant in a gene that is associated with Brugada or long QT syndrome. After the results of genetic testing, we performed additional phenotyping in these patients (*i.e.* reverse phenotyping), but only one of the four variants provided an explanation for the DCM (Table 2). The patient with the LP variant in *DSC2* (c.21dup; p.(Gly8Argfs*23)) had severe left and right ventricular dysfunction, but did not have a definite diagnosis of arrhythmogenic right ventricular dysplasia (ARVD).

The ECG of the patient with the *CACNA1C* variant (c.1097 C > T; p.(Thr366Met)) could not be interpreted for signs of Brugada or long QT as there was biventricular pacing from a CRTD. This patient presented initially with ventricular fibrillation and a severe cardiomyopathy (LVEF 17%). The ECG of the patient with a *KCNQ1* (c.899 C > G; p.(Ala300Gly)) variant indicated signs of unifocal multiple VES (21%) and a left bundle branch block (LBBB) morphology. The ejection fraction was initially above 50% but decreased over time. There were no signs of long QT syndrome in the patient, and no positive family history of cardiomyopathy or arrhythmias. A likely pathogenic variant *SCN3B* variant (c.516 G > A; p.(Trp172*)) was found in a DCM patient, which did not explain her cardiomyopathy (LVEF 43%). There were no signs of Brugada or idiopathic VT, but the ECG indicated a LBBB morphology. There were also no signs of Brugada and long QT syndrome during follow-up of these three patients.

Interestingly, a pathogenic *KCNJ2* (c.224 C > G; p.(Thr75Arg)) variant was reported in one patient. Pathogenic variants in *KCNJ2* are associated with Andersen Tawill syndrome (MIM#170390), a multisystemic channelopathy characterized by periodic paralysis and ventricular arrhythmias. The patient presented initially with torsades de pointes and DCM (LVEF 21%). During follow-up she remained arrhythmogenic and had a decreased LVEF. The variant was de novo and she also showed the phenotypical characteristics such as a small mandibula, low-set ears and clinodactyly. The patient had persistent non-sustained ventricular tachycardias and multifocal ventricular extrasystoles, which possibly contributed to a tachycardia-induced decreased left ventricular ejection fraction and DCM.

Clinical relevance of variants of unknown significance

The majority of detected variants are VUSs that do not lead to clinical actionability, *i.e.* treatment of a patient with DCM will not be changed based on the finding of a VUS, and invasive

Table 2. Clinical characteristics of patients with dilated cardiomyopathy who had a pathogenic or likely pathogenic variant in a gene associated with Brugada or long QT syndrome.

Gene	Electrocardiogram	Structural phenotype	Left ventricular ejection fraction	Initial presentation	Gene variant explanation for phenotype
CACNA1C	Biventricular pacing	DCM	17%	Ventricular fibrillation	No
KCNJ2	Long QT interval and polymorphic ventricular tachycardia	DCM and ATS	21%	OHCA: torsades des pointes	Yes
KCNQ1	Left bundle branch block	DCM	44%	Unifocal ventricular extrasystoles (21%)	No
SCN3B	Left bundle branch block	DCM	43%	Unifocal ventricular extrasystoles (21%)	No

DCM dilated cardiomyopathy, *ATS* Anderson-Tawil syndrome, *OHCA* out of hospital cardiac arrest.

procedures like ICD-implantation is not warranted without the proper understanding of the pathogenicity of a variant. Next, we determined the influence of the number of VUSs detected with the HEART panel on the clinical outcome of patients, therefore we excluded all patients with a P/LP variant ($n = 13$). In total, 91 patients had no variant (43%), and 80, 29, 9 and 3 patients had one (38%), two (14%), three (4%) or four variants (1%) respectively. Although the group of multiple VUSs is small, the number of VUSs was significantly associated with the combined end-point of mortality, heart failure hospitalization, heart transplantation or life-threatening arrhythmias (HR, 1.57 [95% CI, 1.22 to 2.02]; $p < 0.001$; Fig. 3, Table S4 and Table S5). The combination of genes in which a VUSs was detected for each patient can be found in Figure S2 and Table S6.

The same trend was visible when we restrict the detected VUSs to genes in the core panel (Log-rank $p < 0.001$; Figure S3 and Table S5) or robust panel (Log-rank $p < 0.01$; Fig. S4 and Table S5), but not when we included VUSs in genes that were exclusively on the HEART panel (Log-rank $p = 0.2$; Fig. S5). All analyses showed that VUSs have an association with the disease course, although the number of patients in the groups with 3 or 4 VUSs is too small to draw definite conclusions. Therefore, we compared the patients without any genetic variant with the patients with at least one VUS in the HEART panel, core panel, or robust panel (Fig. 4 and Table S5). The presence of a VUS was significantly associated with an adverse outcome (all three analyses $p < 0.05$), but not when we included only VUSs in genes exclusive to the HEART panel ($p = 0.2$; Fig. S5), indicating that the VUSs in the robust panel carry the strongest prognostic value.

DISCUSSION

Using an extensive gene panel for genetic testing of patients with DCM did improve the diagnostic yield very little compared to a gene panel that only includes robust DCM-causing genes. The extensive gene panel did detect multiple VUSs. The presence of a VUS was associated with an increased risk of cardiac mortality and heart failure hospitalization, especially those VUSs in the 14 robust genes.

Constitution of gene panels in current clinical practice

The HEART panel did not increase the clinical sensitivity of detecting monogenic causes in patients with DCM. The expanding number of tested genes led to a significant increase in VUSs which do not alter clinical actionability at this moment, since the evidence for disease association is missing. Therefore, the finding of a VUS does not change the treatment plan of a patient with DCM [4, 16]. Also, we did not find any pathogenic CNVs in these patients. Instead, the finding of VUSs can lead to 'noise' in the diagnostic process as additional evidence has to be sought to proof potential pathogenicity of the variant [14]. However, although the clinical relevance of VUSs are unknown with the current knowledge, the classification can change towards likely pathogenic (or benign) after family segregation. In our study, we had many small families with sporadic DCM, providing no possibility to segregate a detected VUS with the phenotype in the family. Therefore, the HEART panel is not recommended for every patient but is indicated in DCM patients with multiple affected relatives (allowing the possibility for segregation) or in patients with a complex syndrome that includes a cardiac phenotype (*e.g. DCM*). All other patients with DCM should be tested with a small gene panel constituting of only robust DCM genes [8].

Our results are in line with comparable studies in hypertrophic cardiomyopathy (HCM) patients in which the addition of minor genes to genetic testing panels resulted in a small increase of the diagnostic yield and a significant increase of inconclusive results [17, 18]. More than 90 percent of the pathogenic variants in HCM

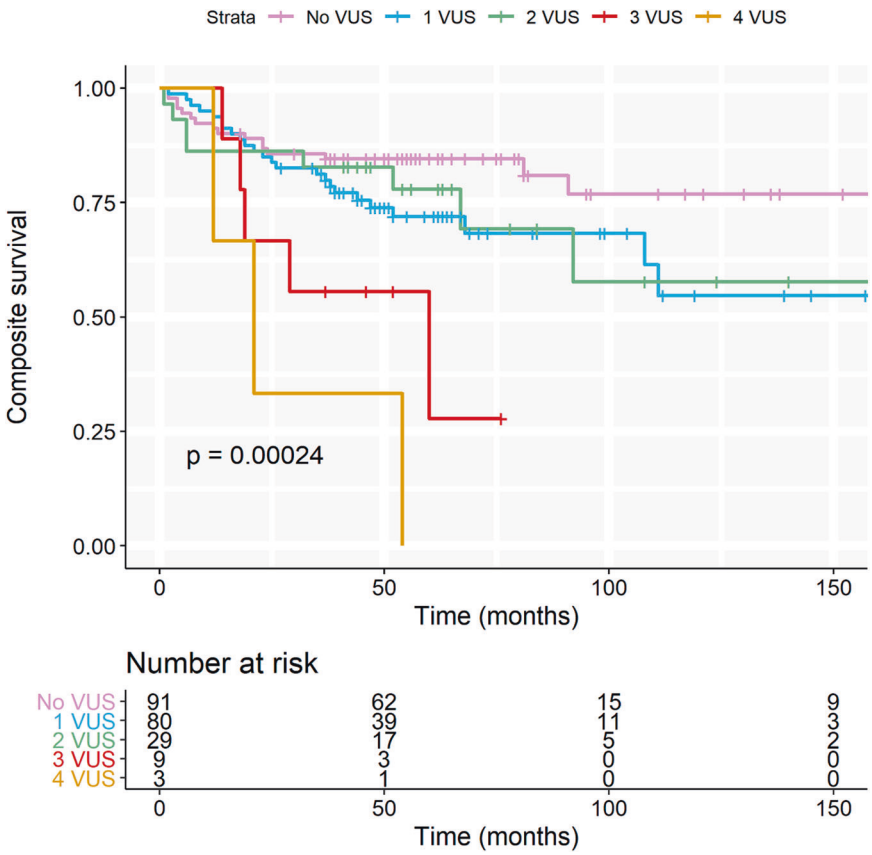

Fig. 3 Survival curves show freedom from combined endpoint (cardiac death or transplantation, heart failure hospitalization or life-threatening arrhythmia) stratified on the number of detected variants of unknown significance (VUSs) in the HEART panel. Patients with dilated cardiomyopathy (DCM) are stratified on the absolute number of VUSs that were identified by the HEART panel (0, 1, 2, 3 or 4 VUSs). Corresponding hazard ratio can be found in Table S5.

patients are found in only eight core genes [10]. Therefore, caution is warranted in using large gene panels in these patients in current clinical practice.

Moreover, we showed that re-evaluation of existing data leads to additional genetic diagnosis in DCM patients without a molecular diagnosis. This is in line with a previous study that performed genetic re-evaluation in 150 pediatric neurology patients which led to an increase of the diagnostic yield of 31% to 53% after 5 years [19]. With growing evidence and knowledge on disease causing variants, genetic re-evaluation should be performed over time for all DCM patients who have received previous genetic testing irrespective of the panel used.

Unsolved families with a familial history of dilated cardiomyopathy

The vast majority of patients with clinically established DCM remain without a genetic diagnosis after testing using the HEART panel, suggestive of other non-genetic causes of DCM [5]. However, in some DCM patients without a detected pathogenic variant, there is a clear familial inheritance pattern. This could indicate that some of the detected VUSs are P/LP variants and associated with DCM in a monogenic inheritance, but can not be classified as such due to the current evidence. Mainly the VUSs in robust DCM-associated genes are suspicious, which is also supported by the fact that DCM patients with a VUS in a robust DCM-associated gene have a worse prognosis compared to DCM patients without genetic variant. This trend is not observed when we only included VUSs in genes that are exclusive to the HEART panel. The worse prognosis is comparable to patients with a P/LP variant [8]. Another explanation for the observed familial inheritance pattern could be that the 'unsolved' families are the result of a polygenic inheritance of DCM in which interactions between rare and common variants (e.g. population broad distributed polymorphisms) in different cardiac and non-cardiac genes contribute to the phenotype [10]. A study including 200,643 individuals from the UK Biobank detected a P/LP variant in a DCM-causing gene in 800 individuals (0.4%), of which 25 individuals were diagnosed with DCM (penetrance of 3.1%) [20]. This study elegantly shows that the prevalence of (likely) pathogenic variants in the general population is relatively high, but the disease penetrance is low in the absence of a familial (polygenic) background. Another study using data from the UK Biobank showed that rare variants in high risk cardiomyopathy genes have a lower disease penetrance when detected in the general population [21]. The reduced disease penetrance of rare pathogenic variants in DCM-causing genes may be explained by familial clustering of polygenic risk that unmasks rare genetic susceptibility. For example, the polygenic background of TTNtv heterozygotes influenced left ventricular volume and function, emphasizing that a heterozygotes' polygenic background influences the penetrance of high impact rare variants [22]. Future studies are necessary to integrate an individuals' polygenic risk into personalized screening strategies.

Additionally, our results indicate an association between the presence of a VUS and a serious adverse event (SAE) such as cardiac death or the risk of hospitalization. This effect remained, even if we only included VUSs from robust DCM-causing genes, indicating that the strongest prognostic signal of VUSs is coming from those genes. Interestingly, patients with HCM and a VUS in a sarcomere gene had a worse prognosis compared to patients with HCM and no variant in the Sarcomeric Human Cardiomyopathy Registry (SHaRe) [23]. The prognostic value of VUSs highlights the

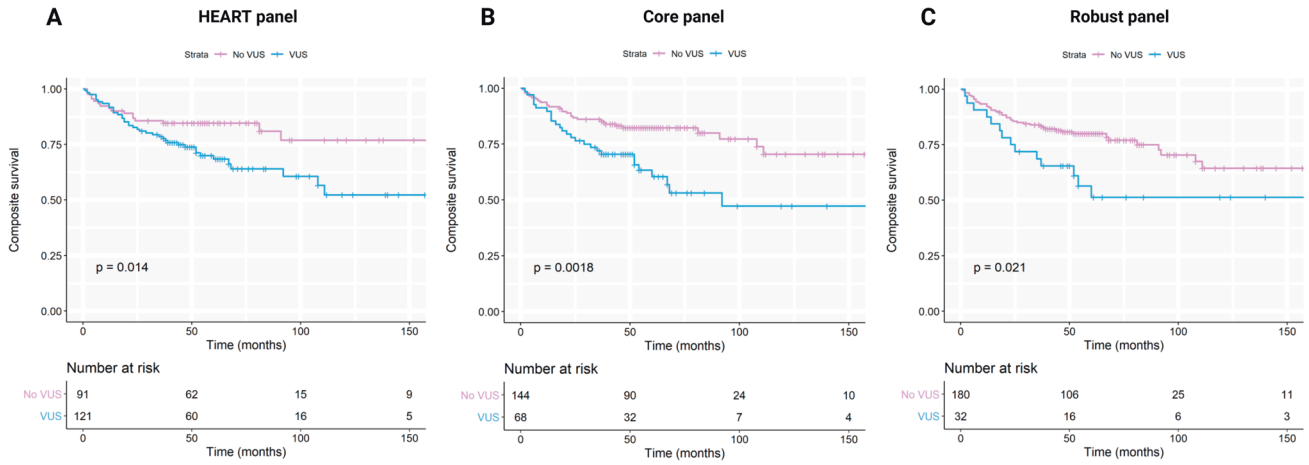

Fig. 4 Survival curves show freedom from combined endpoint (cardiac death or transplantation, heart failure hospitalization or life-threatening arrhythmia) stratified on the presence of at least one variant of unknown significance (VUS). A Patients with dilated cardiomyopathy (DCM) are stratified on the presence of a VUS identified by the HEART panel (*299 genes*), (**B**) the core panel (*48 genes*), or (**C**) the robust panel (*14 genes*). Corresponding hazard ratios can be found in Table S5.

possible contribution of these variants to the disease course of patients with cardiomyopathies.

Genes of unknown significance

The HEART panel constitutes of genes that are associated with cardiac (dys)function in general, and not specific for DCM. Therefore, many genes are of unknown significance (GUS) in patients with DCM. Pathogenic variants in genes associated with Brugada syndrome (BrS) and long QT syndrome (LQT) were found in patients with DCM. These pathogenic variants are not causal for the observed phenotype and therefore do not increase the genetic yield. Additionally, none of these genes are described in GWAS and PRS studies as part of a polygenic cause of DCM. None of the heterozygotes had clinical signs suggestive of BrS or LQT, indicating either low disease penetrance of these genes or an unestablished contribution of these variants to the DCM phenotype. The detected variants in these genes are secondary findings which can be relevant in the follow-up of a patient with already a DCM phenotype. However, although both genes (*SCN3B* and *CACNA1C*) are associated with BrS in OMIM, both genes have been disputed as a causal BrS gene, thereby limiting the clinical actionability [24]. Currently, we do not report VUSs in genes that are not associated with DCM to limit the number of inconclusive results.

Future outlook of genetic testing and clinical recommendations

In total, five VUSs in DCM-causing genes were reclassified to likely pathogenic after reanalysis of the variant in the HEART panel. A recent study reanalyzed exome sequencing data of genetically undiagnosed patients 5 year after first genetic test [19]. A diagnosis was found in 22% of the undiagnosed patients, emphasizing the importance of systematically reanalyzing previous detected VUSs.

The finding of a VUS has no consequences on current clinical management of the patient, but our findings do highlight the effect of a VUS in clinical outcome and highlights the importance that expanding our knowledge can lead to reclassification of these variants in DCM. We also note prognostic differences based on the specific gene in which a VUS was detected, showing the potential of differentiating VUSs on suspicion. Genetic testing in current clinical practice aims to detect a monogenic cause that explains the disease occurrence in families, while limiting the risk of finding uncertain findings. The clinical sensitivity of our HEART panel leading to a molecular diagnosis in patients with DCM is not higher than genetic testing using the smaller core panel. As we have previously shown, even the core panel includes many genes that do not increase the diagnostic sensitivity of a gene panel in a unselected cohort of DCM patients [8]. It is therefore recommended to limit genetic testing in patients with an isolated DCM to the robust genes as curated by the ClinGen consortium [6]. The genetic architecture of DCM is more complex than can be comprehended in current clinical practice. The susceptibility to the disease and the severity of the phenotype are probably subjected to a combination of genetic variants and non-genetic risk factors. It is unlikely that a novel high-penetrant monogenic cause for DCM will be identified, thus the benefit of expanding gene panels for monogenic approaches is not recommended. The challenge for the future of genetic testing in DCM is to integrate the effect size of rare variants in robust genes and variants in minor genes (loci detected by GWAS) into models which are suitable for clinical practice and improve the risk prediction patients and their relatives [25].

CONCLUSION

The use of large gene panels for genetic testing in DCM does not increase the diagnostic yield, although the presence of a VUS was associated with an adverse prognosis of a patient with DCM. Current diagnostic gene panels should be limited to the robust DCM-causing genes.

DATA AVAILABILITY

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

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AUTHOR CONTRIBUTIONS

Conceptualization: SS, DH, JV; Data curation: SS, DH, GC, MH, MS, JV; Formal Analysis: SS, JV; Funding acquisition: JV, HB, A-vdW; Investigation: SS, DH, GC, IK, EV, AH-vdE, JV; Methodology: JV, Resources: HB, A-vdW; Supervision: HB, JV; Visualization: SS, JV; Writing – original draft: SS, DH, JV; Writing – review & editing: GC, IK, MH, MS, EV, AH-vdE.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was performed according to the declaration of Helsinki and was approved by the institutional Medical Ethics Committee of the Maastricht University Medical Center. All patients gave written informed consent.

ADDITIONAL INFORMATION

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