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SHORT COMMUNICATION

Diagnostic yield and predictive value on left ventricular remodelling of genetic testing in dilated cardiomyopathy

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Abstract

Aims We assessed the diagnostic yield of genetic testing and the relationship of left ventricular (LV) reverse remodelling (LVRR) with the presence of DNA pathogenic (P) or likely pathogenic (LP) variants in patients with dilated cardiomyopathy (DCM).

Methods and results From 680 outpatients followed at the Heart Failure Outpatient Clinic of our institution, we selected subjects with a diagnosis of DCM as defined by LV ejection fraction (LVEF) ≤40% and LV dilatation not explained by coronary artery disease or other causes. All patients were offered genetic investigation of 42 disease-associated DCM genes with next-generation sequencing. Seventy patients fulfilled the definition of DCM and 66 underwent genetic investigation. We identified 18 P/LP variants in 16 patients, with a diagnostic yield of 24%. The most common variants were truncating *TTN* variants (n = 7), followed by *LMNA* (n = 3), cytoskeleton Z-disc (n = 3), ion channel (n = 2), motor sarcomeric (n = 2), and desmosomal (n = 1) genes. After a median follow-up of 53 months (inter-quartile range 20–111), patients without P/LP variants exhibited higher systolic and diastolic blood pressure, lower plasma brain natriuretic peptide levels, and a larger extent of LVRR, as reflected by the increase in LVEF (+14% vs. +1%, P = 0.0008) and decrease in indexed LV end-diastolic diameter (−6.5 vs. −2 mm/m^2, P = 0.03) compared with patients with P/LP variants.

Conclusions Our results confirm the high diagnostic yield of genetic testing in selected DCM patients and suggest that identification of P/LP variants in DCM portends poorer LVRR in response to guideline-directed medical therapy.

Keywords Dilated cardiomyopathy; Genetics; Reverse remodelling

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Background

Dilated cardiomyopathy (DCM) is defined by left ventricular (LV) dilatation and systolic dysfunction in the absence of abnormal loading conditions or coronary artery disease as a cause of global systolic impairment.[1] Genetic testing is one essential element in the diagnostic workup of patients with DCM, and genotype–phenotype correlations are still poorly understood.[2] LV reverse remodelling (LVRR) has a protective prognostic role for major cardiovascular events in patients with DCM,[3] and it has been suggested that the identification of a disease-causing gene variant has an impact on LVRR and prognosis in patients with DCM.[4,5]

Aims

The aims of the present study were to assess the diagnostic yield of genetic testing in a selected DCM cohort including

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Table 1 Characteristics of the study population at the time of genetic testing according to presence of pathogenic/likely pathogenic variants

	Total (*n* = 66)	Without P/LP variants (*n* = 50, 76%)	With P/LP variants (*n* = 16, 24%)	*P*
Clinical characteristics				
Age (median, years)	57 (48–65)	59 (50–64)	56 (45–67)	0.61
Male gender (%)	68	70	63	0.58
BMI (kg/m^2)	26.5	26.7	26.0	0.52
Age at DCM diagnosis (median, years)	50 (42–57)	52 (44–58)	45 (45–67)	0.10
History of HHF (%)	68	68	69	0.96
History of atrial fibrillation (%)	26	26	25	0.94
Family history of DCM (%)	35	30	50	0.14
Family history of SCD (%)	20	14	38	**0.04**
NYHA II–III (%)	30	32	25	0.60
Hypertension (%)	35	36	31	0.73
Active/previous smoker (%)	64	66	56	0.48
Alcohol abuse (%)	8	8	6	0.82
sBP (mmHg)	115 ± 16	118 ± 15	108 ± 19	**0.04**
dBP (mmHg)	70 ± 11	72 ± 10	65 ± 10	**0.02**
LBBB (%)	30	36	13	0.08
Blood tests				
eGFR (mL/min/1.73 m^2)	82 ± 21	84 ± 18	77 ± 29	0.28
BNP (median, pmol/mL)	102 (30–231)	55 (25–151)	250 (120–327)	**0.0002**
Echocardiography				
LVEF (%)	39 ± 11	41 ± 10	33 ± 11	**0.006**
LVEDDi (mm/m^2)	31 ± 4	30 ± 4	34 ± 5	**0.003**
Indexed LA diameter (mm/m^2)	22 ± 4	21 ± 3	23 ± 4	0.09
Significant mitral regurgitation (%)	24	18	44	**0.04**
Therapy				
ACEi/ARB (%)	53	54	50	0.78
ARNI (%)	42	44	38	0.65
Beta-blockers (%)	98	100	94	0.07
MRA (%)	68	62	88	0.06
Loop diuretics (%)	55	56	50	0.67
Amiodarone (%)	9	10	6	0.65
CRT (%)	20	24	6	0.12
ICD (%)	39	34	56	0.11

ACEi/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; ARNI, angiotensin receptor–neprilysin inhibitor; BMI, body mass index; CRT, cardiac resynchronization therapy; dBP, diastolic blood pressure; DCM, dilated cardiomyopathy; eGFR, estimated glomerular filtration rate; HHF, hospitalization for heart failure; ICD, implantable cardioverter defibrillator; LA, left atrial; LBBB, left bundle branch block; LVEDDi, indexed left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; sBP, systolic blood pressure; SCD, sudden cardiac death.
Statistically significant *P* values ($P < 0.05$) are in bold.

both familial and nonfamilial cases and to evaluate phenotypic differences and the relationship of LVRR with the presence of DNA pathogenic (P) or likely pathogenic (LP) variants.

Methods

Among 680 patients followed at the Heart Failure Outpatient Clinic of our institution between 2015 and 2021, we selected subjects with a diagnosis of DCM as defined by LV ejection fraction (LVEF) ≤40% and LV dilatation not explained by coronary artery disease or other causes of global systolic dysfunction such as abnormal loading conditions, tachycardiomyopathy, cardiotoxicity, or myocarditis (exclusion criteria are listed in Supporting Information, *Table S1*).[1,6,7] We included DCM patients with a history of alcohol abuse and peripartum cardiomyopathy, which are known to share a genetic predisposition with idiopathic DCM.[8,9]

The time of DCM diagnosis ranged from 1994 to 2021. At the time of the index visit, all patients underwent extensive clinical, laboratory, and imaging evaluation and were offered genetic testing with next-generation sequencing (NGS) of 42 disease-associated DCM genes. We grouped P/LP variants in different gene clusters according to their molecular function and cellular compartment derived from literature (listed in Supporting Information, *Table S2*).[5] Familial cases were ascertained according to published criteria.[10]

To evaluate the impact of guideline-directed medical therapy, we assessed changes in LVEF, indexed LV end-diastolic diameter (LVEDDi), and indexed left atrial diameter as indicators of LVRR between the index visit, defined as the first visit at our centre, and the time of genetic testing. Echocardiography variables for LVRR were measured according to current guidelines.[11]

Genetic testing was performed in the Human Genetic Laboratory of Giannina Gaslini Hospital in Genoa. NGS was performed with the Illumina MiSeq™ system. Coding region and

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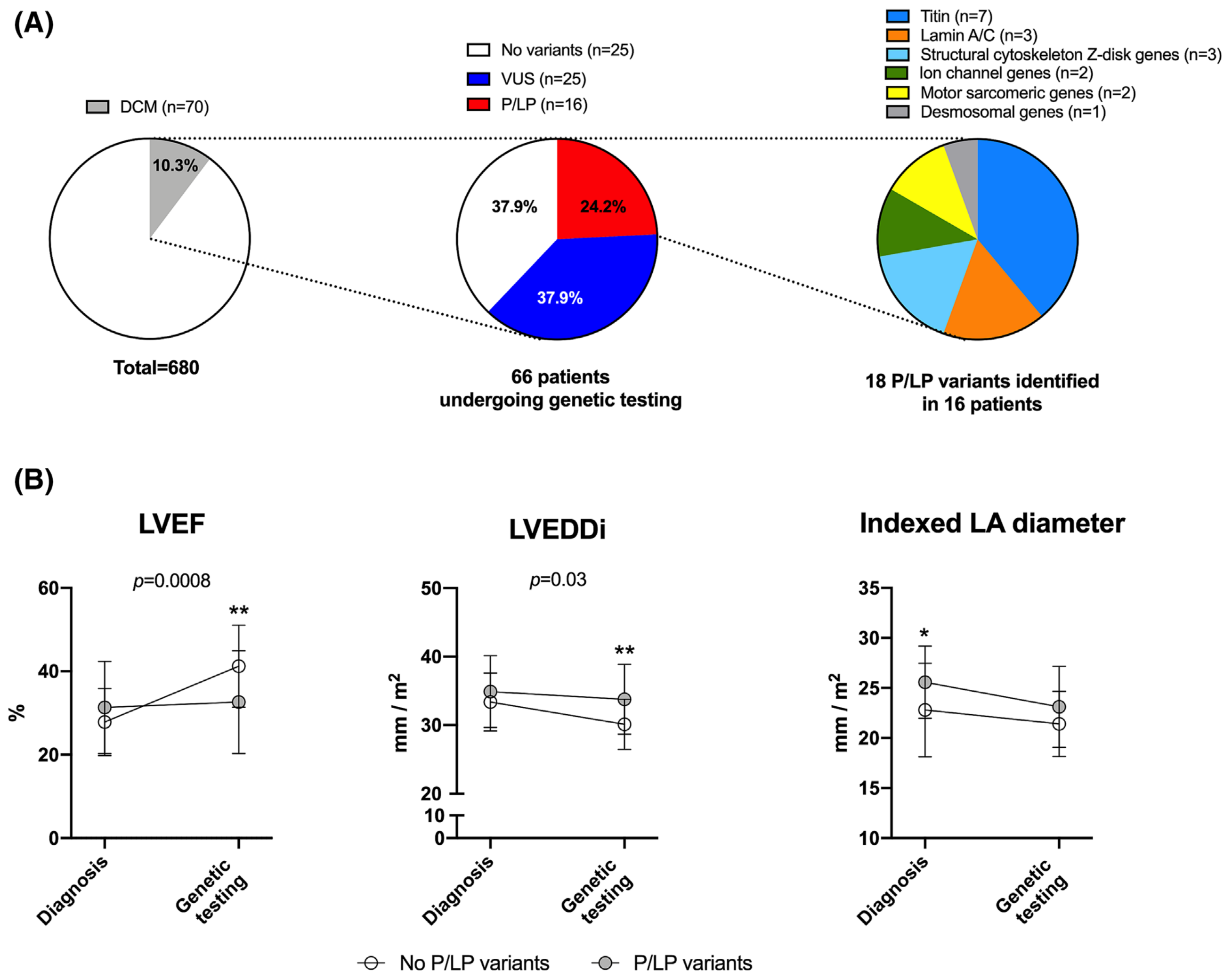
Figure 1 (A) Diagnostic yield of genetic testing and pathogenic/likely pathogenic (P/LP) variants according to gene cluster. (B) Left ventricular reverse remodelling according to the presence of P/LP variants ($n = 16$ patients with P/LP variants; $n = 50$ non-carriers). In (B), the *P* value indicates a significant interaction between time and genotype on left ventricular ejection fraction (LVEF) and indexed left ventricular end-diastolic diameter (LVEDDi) by two-way ANOVA; '*' and '**' indicate significant ($P < 0.05$ and $P < 0.01$, respectively) differences in LVEF and LVEDDi at the time of genetic testing patients with and without P/LP variants by Bonferroni *post hoc* test. DCM, dilated cardiomyopathy; LA, left atrial; VUS, variant of unknown significance.



exon–intron junctions (five nucleotides) of the target genes were enriched using a customized kit purchased from Sophia Genetics. The coding DNA sequences and the exon–intron junctions were examined with a minimum analysis depth of 20× and a 99% coverage of the target region. Sanger sequencing (sensitivity and specificity of 99%) was used to confirm P/LP variants. The human reference genome hg19 was used for sequence alignment. Primary analysis and variant calling were performed by Sophia DDM platform. Interpretation and classification of variants were performed by consulting international databases such as Human Gene Mutation Database (HGMD) and ClinVar and bioinformatics tools such as Alamut and Franklin and according to guidelines and recommendations updated to 31 May 2022.[12–14]

This study conforms to the principles outlined in the Declaration of Helsinki.[15]

All values are reported as mean ± standard deviation, median and inter-quartile range (IQR), or percentages. Continuous variables were compared by Student's *t*-test, and the χ^2 test was used for binary variables. Changes in parameters of LVRR over time were compared by two-way ANOVA followed by Bonferroni's multiple comparisons test. Statistical analysis was performed using Prism Version 8.4.0.

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Results

Seventy patients fulfilled the definition of DCM and 66 gave consent to undergo genetic investigation. Demographic and clinical characteristics at the time of genetic testing are summarized in *Table 1*. The median age at diagnosis was 50 years (IQR 42–57). Twenty-nine (44%) patients had a family history of DCM and/or sudden cardiac death. We included five patients with a history of alcohol abuse. We identified 18 P/LP variants in 16 patients, of whom 2 patients carried 2 P/LP variants (a complete list and classification of P/LP variants can be found in Supporting Information, *Table S3*). Furthermore, 25 patients carried variant of unknown significance (VUS) (*Figure 1A*). The most common P/LP variants were truncating *TTN* gene variants (n = 7, 39% of all P/LP variants), followed by *LMNA* (n = 3, all missense variants), cytoskeleton Z-disc (n = 3, 2 missense variants and 1 truncating variant), ion channel (n = 2, all missense variants), motor sarcomeric (n = 2, all missense variants), and desmosomal (n = 1, truncating) genes (*Figure 1A*). Overall, genetic diagnostic yield was 24%.

At the time of DCM diagnosis, patients carrying P/LP variants tended to have a higher LVEF compared with subjects without (31 ± 11% vs. 28 ± 8%, P = 0.17). The median follow-up time between DCM diagnosis and genetic testing at our centre was 53 months (IQR 20–111) and was longer for patients carrying one P/LP variant with respect to non-carriers [94 (IQR 20–111) vs. 39 months (IQR 14–83), P = 0.01]. At the time of genetic testing, the majority of patients were in New York Heart Association (NYHA) Class I, and all patients had received optimal medical therapy for at least 12 months, with the vast majority receiving the highest tolerated dose of angiotensin-converting enzyme (ACE) inhibitors/angiotensin receptor blockers or sacubitril/valsartan (95%) together with beta-blockers (98%). Despite similar optimal guideline-directed medical therapy, patients with P/LP variants had lower systolic and diastolic blood pressure, lower LVEF, larger LVEDDi, and higher plasma brain natriuretic peptide (BNP) levels compared with patients without P/LP variants (*Table 1*). In addition, the prevalence of moderate or severe mitral regurgitation tended to be higher among carriers of P/LP variants. Patients without P/LP variants exhibited a larger extent of LVRR between DCM diagnosis and genetic testing, as reflected by the larger increase in LVEF (+14% vs. +1%, P for an interaction between time and genotype 0.0008) and decrease in LVEDDi (-3 vs. -1 mm/m^2, P = 0.03) compared with patients with P/LP variants (*Figure 1B*). The results remained unchanged when patients with a history of alcohol/drug abuse (n = 5) and peripartum cardiomyopathy (n = 1) were excluded from the analysis (Supporting Information, *Figure S1*) and when comparing 16 patients carrying P/LP variants with 16 non-carriers matched for follow-up duration [Supporting Information, *Figure S2*, median follow-up duration 81 (IQR 57–153) and 93 months (IQR 59–141) for non-carriers and P/LP carriers, respectively, P = 0.93].

Two patients carrying P/LP variants in the *DSP* and *DMD* genes, respectively, underwent heart transplantation following genetic testing.

Conclusions

The results of this study confirm the importance of genetic testing to optimize the prediction of LVRR in DCM patients in response to guideline-directed medical therapy. In our cohort, genetic testing had a diagnostic yield of 24%, slightly lower than reported previously,[2,5] and identified *TTN* truncating variants as the most common P/LP variants in DCM patients, consistent with other studies.[2,6]

At the index visit at our centre, P/LP carriers exhibited a higher LVEF compared with non-carriers, and all patients were started on optimal medical therapy that was progressively titrated up to the maximal tolerated dose. Nevertheless, after a median follow-up of 53 months, patients with one P/LP variant had lower systolic and diastolic blood pressure and higher BNP levels and exhibited a lower extent of LVRR compared with patients without P/LP variants. Subsequently, two patients carrying one desmosomal (*DSP*) and one cytoskeleton Z-disc (*DMD*) gene variants, respectively, underwent heart transplantation. These results are in line with previous studies indicating that the identification of P/LP variants is associated with a lower rate of LVRR,[5] which is a strong prognostic predictor in DCM.[2,3] Altogether, these observations suggest that neurohormonal inhibition is not sufficient to halt maladaptive remodelling in the presence of gene variants causing primary cardiac myocyte dysfunction.

Important shortcomings of this analysis must be acknowledged. First, because of the small number of patients carrying P/LP variants, we analysed LVRR in all P/LP carriers vs. non-carriers rather than differentiating between different gene clusters. Previous studies from larger cohorts indicate that different genetic substrates are associated with opposing effects on LVRR; specifically, *TTN* truncating variants are associated with a higher rate of LVRR, whereas *LMNA* or desmosomal variants tend to exhibit a lower rate of LVRR.[4,5,16] Furthermore, we selected inclusion and exclusion criteria for our study based on those adopted by the largest DCM study performed thus far[6]; however, recent evidence indicates that also patients with myocarditis and chemotherapy-related cardiotoxicity, whom we excluded from our study cohort, share a genetic predisposition with idiopathic DCM.[17] Therefore, we acknowledge a possible selection bias of our study cohort.

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Altogether, our results confirm the diagnostic yield of genetic testing in selected DCM patients and suggest that identification of P/LP variants in DCM portends poorer LVRR in response to guideline-directed medical therapy.

Conflict of interest

None declared.

Funding

None.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1: Exclusion criteria.
Table S2: Gene clusters.
Table S3: Pathogenic and likely pathogenic variants identified.
Figure S1: Left ventricular reverse remodeling according to the presence of pathogenic/likely pathogenic variants excluding patients with a history of alcohol/drug abuse (n = 5) and peripartum cardiomyopathy (n = 1).
Figure S2: Left ventricular reverse remodeling in patients with pathogenic/likely pathogenic (P/LP) variants (n = 16) and non-carriers matched 1:1 based on follow-up duration.

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