Genetic Risk of Arrhythmic Phenotypes in Patients With Dilated Cardiomyopathy

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ABSTRACT

BACKGROUND Genotype-phenotype correlations in dilated cardiomyopathy (DCM) and, in particular, the effects of gene variants on clinical outcomes remain poorly understood.

OBJECTIVES The purpose of this study was to investigate the prognostic role of genetic variant carrier status in a large cohort of DCM patients.

METHODS A total of 487 DCM patients were analyzed by next-generation sequencing and categorized the disease genes into functional gene groups. The following composite outcome measures were assessed: 1) all-cause mortality; 2) heart failure–related death, heart transplantation, or destination left ventricular assist device implantation (DHF/HTx/VAD); and 3) sudden cardiac death/sustained ventricular tachycardia/ventricular fibrillation (SCD/VT/VF).

RESULTS A total of 183 pathogenic/likely pathogenic variants were found in 178 patients (37%): 54 (11%) *Titin*; 19 (4%) Lamin A/C (*LMNA)*; 24 (5%) structural cytoskeleton-Z disk genes; 16 (3.5%) desmosomal genes; 46 (9.5%) sarcomeric genes; 8 (1.6%) ion channel genes; and 11 (2.5%) other genes. All-cause mortality was no different between variant carriers and noncarriers ($p = 0.99$). A trend toward worse SCD/VT/VF ($p = 0.062$) and DHF/HTx/VAD ($p = 0.061$) was found in carriers. Carriers of desmosomal and *LMNA* variants experienced the highest rate of SCD/VT/VF, which was independent of the left ventricular ejection fraction.

CONCLUSIONS Desmosomal and *LMNA* gene variants identify the subset of DCM patients who are at greatest risk for SCD and life-threatening ventricular arrhythmias, regardless of the left ventricular ejection fraction.

Dilated cardiomyopathy (DCM) is a primary heart muscle disease, characterized by left ventricular (LV) dilation and progressive systolic dysfunction (1). Despite an improvement in prognosis over the last several decades, DCM remains a major cause of heart failure (HF) and is the leading cause of cardiac transplantation. With the advent of high-quality next-generation sequencing (NGS) extended panels, the genetic causes of DCM have been increasingly identified (2). More than 50 genes

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are currently considered to be disease-related, and causative variants can be identified in approximately 20% to 50% of all DCM cases (2). Titin (*TTN*) is the most common gene involved with truncation variants accounting for 19% to 25% of familial and 11% to 18% of sporadic forms (3). Other disease-related gene groups implicated in the pathogenesis include those encoding the sarcomere/Z-band, desmosome, cytoskeleton, nuclear lamina, mitochondria, and calcium/sodium handling proteins (4). Although some data suggest that variants in specific genes (i.e., *LMNA* and *FLNC* mutations) are associated with increased arrhythmic risk (5,6), large-scale genotype-phenotype correlation studies including outcome comparisons for patients carrying different disease-related genes variants are limited. In the present study, we sought to assess and compare the effects of variants belonging to different functional gene groups on long-term outcomes in a large cohort of DCM patients.

METHODS

STUDY POPULATION. We analyzed all DCM patients with available NGS data enrolled from January 1, 1988, to December 31, 2015, in the Familial Cardiomyopathy Registry, which is a multicenter (i.e., Cardiovascular Department, University of Trieste, Italy and Cardiovascular Institute, University of Colorado Anschutz Medical Campus, Aurora, Colorado) ongoing project studying hereditary human cardiomyopathies (Online Appendix). The diagnosis of DCM was performed in the presence of left ventricular ejection fraction (LVEF) <50% at the time of diagnosis in the absence of any known possible cause of left ventricular dysfunction (1,7,8). It is noteworthy that all of the patients fulfilling criteria for "definite," "probable," or "possible" arrhythmogenic right ventricular cardiomyopathy (ARVC) (with the exception of desmosomal mutation carrier status) according to 2010 Task Force diagnostic Criteria of ARVC (9) have been excluded from the study to avoid the inclusion of patients with phenotypic ARVC. Family history was extensively investigated, and a ≥3-generation pedigree was constructed; all familial cases fulfilled the published criteria of 2 or more affected individuals in a single family or unexplained sudden death in a first-degree relative of a DCM patient (10).

Echocardiographic LV dimensions and LV and right ventricular systolic function were assessed as currently recommended by international guidelines (11).

STUDY OUTCOME MEASURES. The study outcome measures were: 1) all-cause mortality; 2) heart failure death, heart transplantation for refractory heart failure, or destination therapy left ventricular assist device implantation for refractory heart failure (DHF/HTx/VAD); and 3) sudden cardiac death/sustained ventricular tachycardia/ventricular fibrillation (SCD/VT/VF). Sudden cardiac death (SCD) was defined as witnessed SCD with or without documented ventricular fibrillation (VF), death within 1 h of acute symptoms, or nocturnal death with no antecedent history of immediate worsening symptoms. Ventricular tachycardia (VT)/VF were defined as VF, sustained VT (i.e., lasting ≥30 s or with hemodynamic instability), appropriate implantable cardioverter-defibrillator (ICD) interventions (shock or antitachycardia pacing) on VF, or sustained VT ≥185 beats/min. The outcome status of the patients was obtained through extensive contact of civic registries, families, and general practitioners for patients without recent clinical evaluation. The follow-up ended at the date of the endpoints experience, at the last available contact with the patient, or on December 31, 2016. No patients included in the study were lost to follow-up.

GENETIC ANALYSIS. Patient samples were screened using NGS for variants in 23 well-established causative (disease-related) genes. Details of platforms and filtering processes used in this study are found in the Online Appendix. These 23 genes were selected because they account for >97% of genetic cases of DCM according to our data and available evidence from the published data (2), and encompass all mutations detected by our clinical platforms (>100 DCM gene panels) in over 100 patients. Analyzed genes included *ACTC1, BAG3, DES, DMD, DSC2, DSG2, DSP, FLNC, JUP, LAMA4, LDB3, LMNA, MYBPC3, MYH6, MYH7, NEXN, PKP2, RBM20, RYR2, SCN5A, TMEM43, TNNT2,* and *TTN*. All variants were validated with bidirectional Sanger sequencing, and only variants classified as "pathogenic" or "likely pathogenic" according to the American College of Medical Genetics criteria (12), as described in the Online Appendix, were considered for this analysis. To maintain a conservative approach, all variants of uncertain significance were excluded from the analysis. When available, additional affected family members were used for segregation analysis, and variants that did not cosegregate in all affected family members were not considered. Detailed lists of identified

ABBREVIATIONS AND ACRONYMS

ARVC = arrhythmogenic right ventricular cardiomyopathy

DCM = dilated cardiomyopathy

DHF/HTx/VAD = heart failure–related death/heart transplantation/implantation of left ventricular assist device

HF = heart failure

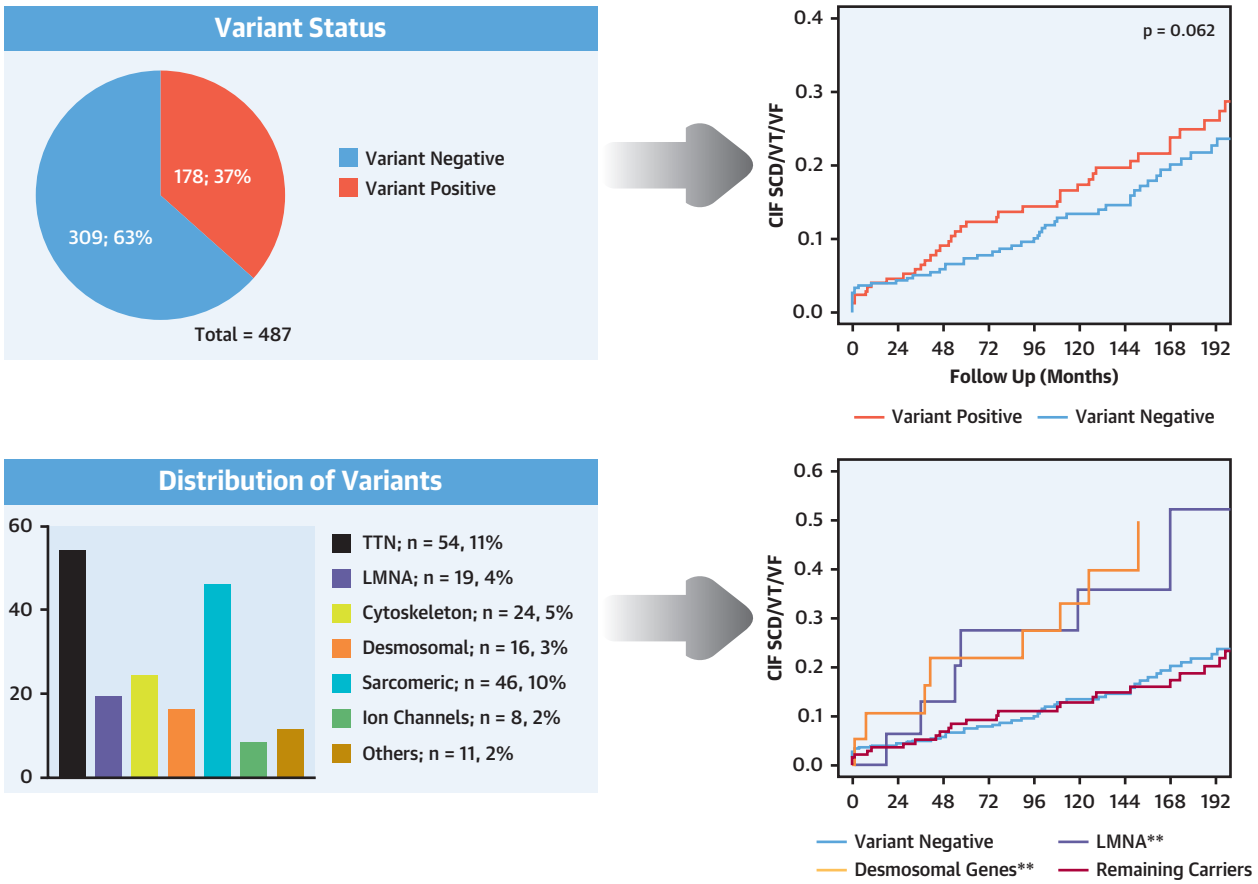
ICD = Implantable cardioverter-defibrillator

LVEF = left ventricular ejection fraction

NGS = next-generation sequencing

SCD/VT/VF = sudden cardiac death/sustained ventricular tachycardia/ventricular fibrillation

CENTRAL ILLUSTRATION **Effect of Genotype on Outcome in Dilated Cardiomyopathy**



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(Left top) Distribution of pathogenic/likely pathogenic variants in the overall study cohort. **(Left bottom)** Distribution of pathogenic/likely pathogenic variants in the study cohort according to each functional gene cluster. **(Right top)** Cumulative incidence curves for SCD/VT/VF in variant-positive versus variant-negative. **(Right bottom)** Kaplan-Meier survival curves for SCD/VT/VF in desmosomal and Lamin A/C (*LMNA*) variant carriers compared with the other patients. The yield of genetic testing in our cohort was 37%. Titin (*TTN*) was most frequently identified (n = 54, 11% of the total population), whereas *LMNA* and desmosomal variant carriers were respectively 19 (4%) and 16 (3%). Variant-positive patients carried a borderline higher arrhythmic risk compared with variant-negative (p = 0.062). Among different genetic clusters, desmosomal variants **(orange line)** experienced a similar rate of SCD/VT/VF compared with *LMNA* variants (**purple line**; p = NS) and higher compared with variant-negative (**blue line**; p = 0.006) and to the remaining carriers (**dark red line**; p = 0.015). CIF = cumulative incidence function; SCD/VT/VF= sudden cardiac death/ventricular tachycardia/ventricular fibrillation.

pathogenic/likely pathogenic variants, data filtering procedures, interpretations, and genetic classification methods are described in the Online Appendix.

GENETIC-BASED CLASSIFICATIONS AND FUNCTIONAL GENE GROUPS. The 23 causative genes analyzed were grouped into functional gene groups as previously described based on similar common gene ontology (GO) functions, involvement in biological processes, localization to subcellular compartments, and other shared properties based on consolidated scientific evidence from the published data and available biological databases (13-15). In this way, 21 genes were assigned to 1 of the following resultant functional gene groups: sarcomeric genes, structural cytoskeleton-Z disk genes, desmosomal genes, ion channel genes, and other genes. *TTN* and *LMNA* were considered as separate groups, due to their specific characteristics of frequency and phenotype in DCM. *MYH7, ACTC1, TNNT2, MYH6,* and *MYBPC3* were included in the sarcomeric genes group. Each of these genes encode for components of thick and thin

sarcomeric filaments and are involved in sarcomeric contraction, sharing the GO molecular functions of "catalytic activity" and "actin binding motor protein" (GO 0000146; GO 0003824). *DES, DMD, FLNC, NEXN,* and *LDB3* were included in the structural cytoskeleton-Z disk genes, merging both sarcolemmal and sarcoplasmatic cytoskeletal protein-coding genes. *PKP2, DSC2, DSP, DSG2,* and *JUP* were grouped together as desmosomal genes (16,17). *RYR2* and *SCN5A* were included in the ion channel genes group. Patients harboring variants in the remaining screened genes were grouped in an "other genes" group, including *TMEM43*, *RBM20*, *BAG3*, and *LAMA4*.

STATISTICAL ANALYSIS. Clinical and laboratory statistics are reported as means and SDs, medians and interquartile ranges, or counts and percentages, as appropriate. Cross-sectional comparisons between groups were made by the analysis of variance test on continuous variables, using the Brown-Forsythe statistic when the assumption of equal variances did not hold, or the nonparametric Mann-Whitney *U* test when necessary. The chi-square or Fisher exact tests were calculated for discrete variables. Survival curves were estimated using follow-up from diagnosis. Cumulative incidence curves of competing events of DHF/HTx/VAD versus SCD/VT/VF were also estimated and compared between groups. We also calculated the survival and the cumulative incidence curves from birth date that are reported in Online Appendix. Because patients were not followed from birth, but instead they entered into the study cohort at different ages, appropriate survival methods taking into account left truncation were used (18,19). As some patients were grouped as families, family clustering was taken into account: when comparing the survival curves, we reported p values derived from Cox regression models with "variant positive versus negative" or "type of variant" as a factor and the family code as a cluster indicator. The R statistical package version 3.4.0 (R Foundation, Vienna, Austria) was used, with libraries "survival," "etm," and "mstate."

RESULTS

SPECTRUM OF DCM GENES. A total of 487 DCM patients, including 429 probands (88%), were analyzed: 329 (68%) U.S. patients and 158 (32%) Italian patients. A total of 183 disease-related pathogenic or likely pathogenic variants were identified in 178 patients (37%), with prevalence significantly higher in familial (43%) than in sporadic cases (27%; $p < 0.001$) (**Central Illustration**, left upper panel, **Table 1**) (see Online Table 1 for the complete list of the variants).

TABLE 1 Diagnostic Yield and Genetic Distribution of Functional Gene Groups Among Familial and Sporadic Cases

	All (N = 487)	Sporadic (n = 194)	Familial (n = 293)
Patients			
Female	158 (32)	46 (24)	112 (38)
Male	329 (68)	148 (76)	181 (62)
Total	487 (100)	194 (40)	293 (60)
Diagnostic yield			
Pathogenic, likely pathogenic			
Variant-positive	178 (37)	52 (27)	126 (43)
Variant-negative	309 (63)	142 (73)	167 (57)
Functional gene groups			
Titin (*TTN*)	54 (11)	14 (26)	40 (74)
Lamin A/C (*LMNA*)	19 (4)	5(26)	14 (74)
Structural cytoskeleton-Z-disk genes (*FLNC, DES, DMD, NEXIN, LDB3**)	24 (5)	9 (38)	15 (62)
Desmosomal genes (*DSP, DSC2, DSG2, PKP2, JUP**)	16 (3)	6 (37)	10 (63)
Motor sarcomeric genes (*MYBPC3, MYH6, MYH7, TNNT2, ACTC1**)	46 (10)	13 (28)	33 (72)
Ion channel genes *(SCN5A, RYR2)*	8 (2)	2 (25)	6 (75)
Other genes (*TMEM43, RBM20, BAG3, LAMA4*)	11 (2)	3 (27)	8 (73)

Values are n (%). *No mutations found in these genes.

Five patients had 2 pathogenetic/likely pathogenetic variants on different genes or affecting the same gene (1.0%), and 3 of them had poor outcomes: 1 died of progressive HF, 1 underwent heart transplantation, and 1 with double desmosomal mutation died suddenly.

The most frequently involved gene was *TTN* (11%, 54 patients with different truncating variants affecting cardiac isoforms N2B and N2BA), followed by *TNNT2* (6%, 28 patients with truncating and missense variants), *LMNA* (4%, 19 patients with truncating and missense variants), *DSP* (3%, 14 patients with truncating and missense variants), and *FLNC* (2%, 12 patients with truncating variants). The remaining 13 genes (*MYBPC3, SCN5A, DMD, DES, MYH7, RBM20, LAMA4, NEXN, PKP2, DSC2, TMEM43, BAG3,* and *RYR2*) accounted for a lower frequency (<2%) (**Table 1**, Online Table 1). Therefore, according to the proposed classification, the following functional gene groups were identified: 1) 54 (11%) *TTN* carriers; 2) 19 (4%) *LMNA* carriers; 3) 24 (5%) structural cytoskeleton-Z disk gene carriers; 4) 16 (3%) desmosomal genes carriers; 5) 46 (10%) sarcomeric carriers; 6) 8 (2%) ion channels; 7) 11 (2%) "other genes"; and 309 (63%) "no/unknown mutation" (**Central Illustration**, left bottom panel, **Table 1**) (see Online Figure 1 for the prevalence of mutations clusters in probands).

STUDY POPULATION. **Table 2** shows the main demographic and clinical characteristics at enrollment

TABLE 2 Comparison of Baseline Characteristics of the Study Population by Mutation Status

	Total (N = 487)	Variant-Positive (n = 178; 37%)	Variant-Negative (n = 309; 63%)	p Value
Age, yrs	41 ± 14	40 ± 15	42 ± 13	0.095
Male	68	67	68	0.802
Caucasian	94	98	92	0.009
NYHA functional class III to IV	27	21	30	0.048
Familial DCM	60	71	54	<0.001
AF	7	7	7	0.948
LBBB	23	14	27	0.001
NSVT	30	35	27	0.057
PVCs ≥100/24 h	26	28	24	0.353
≥50 couplets/24 h	7	6	8	0.514
AV blocks	6	8	5	0.176
LVEDD, mm	65 ± 11	63 ± 10	65 ± 11	0.099
LVEF, %	33 ± 12	33 ± 11	32 ± 13	0.366
RVD	23	25	22	0.708
ACE inhibitors/ARBs	86	88	85	0.438
Beta-blockers	88	86	90	0.223
CRT	16	11	18	0.036
ICD	40	46	37	0.052

Values are mean ± SD or %.

ACE = angiotensin-converting enzyme; ARBs = angiotensin receptor blockers; AV = atrioventricular; CRT = cardiac resynchronization therapy; DCM = dilated cardiomyopathy; ICD = implantable cardioverter-defibrillator; LBBB = left bundle branch block; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; NSVT = nonsustained ventricular tachycardia; NYHA = New York Heart Association functional class; PVCs = premature ventricular complex; RVD = right ventricular dysfunction.

of the total study cohort divided by variant-positive and variant-negative. Online Table 2 shows the main enrollment features according to functional gene group classification. Mean age was 41 ± 14 years, 68% were male, and 94% were Caucasians. Mean LVEF was 33 ± 12%, 27% were highly symptomatic (i.e., New York Heart Association functional class ≥III); 23% presented with right ventricular dysfunction (i.e., right ventricular fractional area change <35% and/or tricuspid annular plane systolic excursion <17 mm). Treatment included beta-blockers and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers in 88% and 86%, respectively, while 40% received an ICD and 16% cardiac resynchronization therapy associated with ICD. Mutations were more common in Caucasians, whereas patients without mutations were more symptomatic, more likely had left bundle-branch block, and received cardiac resynchronization therapy.

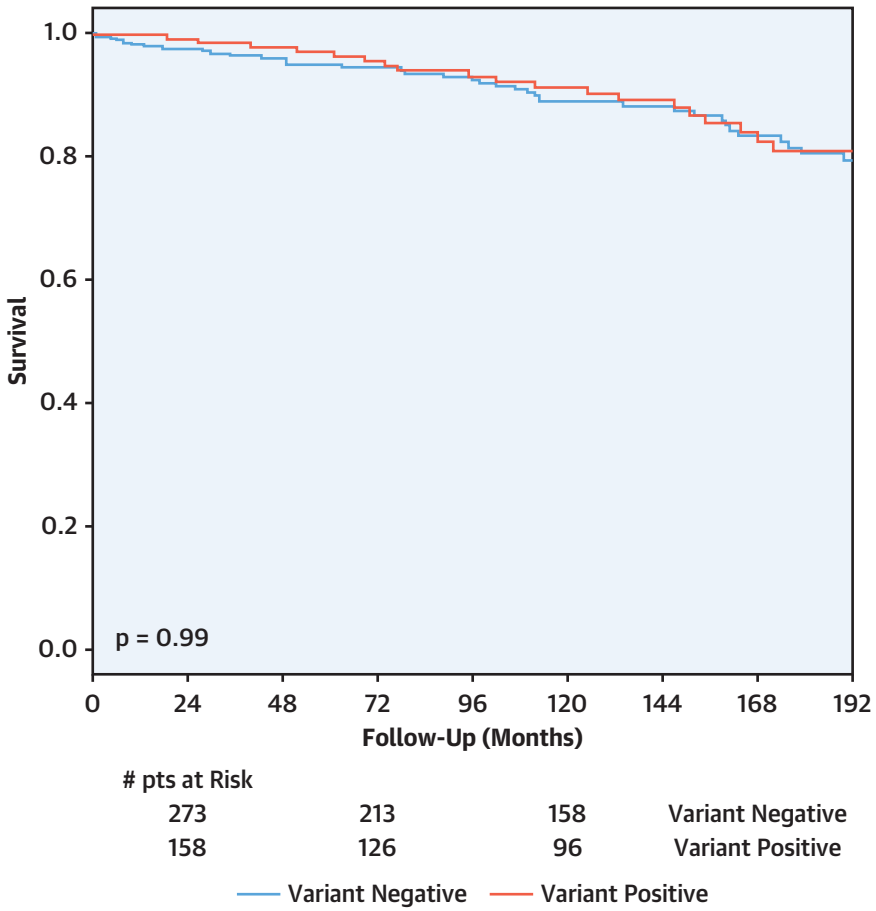
OUTCOMES. Over a median follow-up of 125 months (10.4 years) (range 54 to 185 months), 131 patients died, (27.5%, 2.5 events/100 patient-years), 105 experienced DHF/HTx/VAD (21.5%, 2.2 events/100 patient-years) and 98 experienced SCD/VT/VF (20.1%, 1.8 events/100 patient-years). Among arrhythmia-related events, 10 (2%, 0.2 events/100 patient-years) were SCD, 30 (6%, 0.6 events/100 patient-years) ventricular fibrillation or sustained ventricular tachycardia, and finally, 58 (12%, 1.0 events/100 patient-years) were appropriate ICD interventions. Kaplan-Meier curve analysis showed similar overall survival between variant-positive and variant-negative (p = 0.99) (**Figure 1**). Notably, at competing risk analysis, a strong trend toward a higher cumulative incidence of DHF/HTx/VAD (p = 0.061) (**Figure 2**) and of SCD/VT/VF (p = 0.062) (**Central Illustration**, right upper panel) was observed in variant carriers compared with noncarriers. The same findings were confirmed at competing risk survival analysis starting from birth (Online Figure 2), with stronger differences between carriers and noncarriers for SCD/VT/VF (p = 0.02).

DESMOSOMAL VARIANTS AND RISK OF MAJOR VENTRICULAR ARRHYTHMIAS. Table 3 summarizes the rates of outcome measures analyzed separately according to the functional gene groups. Compared with variant-negative patients and other variant carriers, the *LMNA* demonstrated a higher occurrence of both the DHF/HTx/VAD (**Figure 3**, Online Figure 3) (p < 0.001) and SCD/VT/VF (**Central Illustration**, right bottom panel, Online Figure 3) (p = 0.002 vs. variant-negative and p = 0.003 vs. remaining carriers). Interestingly, carriers of desmosomal variants were also characterized by a significantly higher rate of arrhythmic events compared with both variant-negative patients (p = 0.006) and remaining carriers (p = 0.015), with a risk that was comparable to the *LMNA* subgroup (**Central Illustration**, right bottom panel, Online Figure 3). Desmosomal mutation group presented RV systolic dysfunction in 13% of cases, and mean left ventricular ejection fraction was 32 ± 10% (Online Table 2). However, as shown in **Figure 4**, the arrhythmia was not related to the severity of LV dysfunction, as the survival curves were similar for patients with an LVEF >35% or ≤35% (p = 0.79). According to inclusion and exclusion criteria, none of them had regional right ventricular akinesia, dyskinesia, or aneurysm, which are criteria for ARVC diagnosis.

DISCUSSION

In this study, we report that different genotypes have different outcomes in DCM: indeed, we show that rare

FIGURE 1 Survival Kaplan-Meier Curves in Variant-Positive Versus Variant-Negative Patients

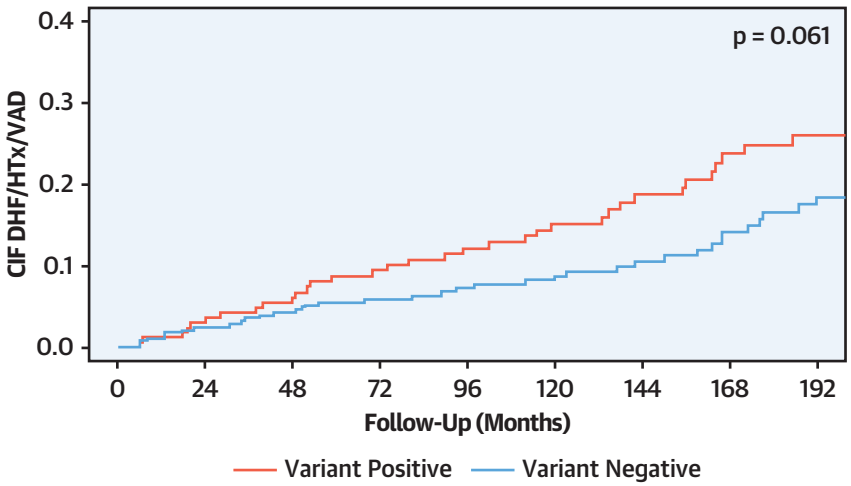


Variant-positive **(red line)** versus variant-negative patients **(blue line).** Survival rate was not different according to variant status (p = 0.99).

variants in desmosome genes, in addition to the known effect of *LMNA* variants (5), define subpopulations with high risk of life-threatening arrhythmias (SCD/VT/VF), irrespective of severity of LV dysfunction. We also found that a positive genotype was associated with a trend toward a worse outcome. Our findings are based on a large study cohort with extensive phenotype characterization, genotyped by next-generation sequencing, and with a median follow up of 10 years. The present study confirms the high diagnostic yield of NGS in a large and well-characterized cohort of patients with DCM. More variants were identified in patients with positive family history compared with sporadic cases (43% vs. 27%, respectively), and multiple correlations were identified between carrier status and adverse clinical outcomes.

GENETICS OF DCM. Pathogenic variants were identified in 37% of our study population, confirming the significant genetic yield in DCM patients achieved by NGS technologies (2). *TTN* was the most affected gene (11%), consistent with most recent published data (2,20). As previously reported (15), the "functional gene group" genotype classification adopted in this study was used to translate the results of genetic testing into information on prognosis, potentially affecting clinical decision-making. The results in this DCM population confirmed the negative effect of *LMNA* variants, but in addition, demonstrated that desmosomal variants are associated with an increased risk of SCD/VT/VF, which is comparable to *LMNA* variants. The presence of a subgroup of patients with DCM carrying desmosomal gene mutations have been previously reported (2,21,22), and

FIGURE 2 Cumulative Incidence Curves for DHF/HTX/VAD in Variant-Positive Versus Variant-Negative



Variant-positive **(red line)** versus variant-negative **(blue line).** Mutation carriers exhibited a borderline higher rate of DHF/HTx/VAD ($p = 0.061$). CIF = cumulative incidence function; DHF/HTx/VAD = death for heart failure/heart transplant/ventricular assist device.

DSP carrier status in ARVC has been described to be associated with left-dominant and biventricular phenotypes (23). A recent large autopsy series with clinical correlation confirms that LV involvement is present in the majority of cases of ARVC and that LV involvement may often be clinically diagnosed as DCM rather than arrhythmogenic cardiomyopathy, yet harbor desmosomal variants (24). In our study, we confirmed that desmosomal variants are relatively common in patients presenting with a DCM phenotype (i.e., 3%). Of note, to avoid the inclusion of confounding phenotypes, all patients with diagnostic phenotypic criteria for definite, probable, or possible ARVC were excluded by protocol (9). The manifestation of desmosomal mutations with pure DCM phenotype highlights the frequent genotypic overlap between DCM and ARVC and suggests to consider them under the wider spectrum of "arrhythmogenic cardiomyopathy" (**Figure 5**). DCM and ARVC may share several features, including evidence of familial disease, pathology (fibrofatty infiltration of the right ventricular wall), ECG features (T-wave inversion in antero-lateral leads), and biventricular involvement (**Figure 5**) (25).

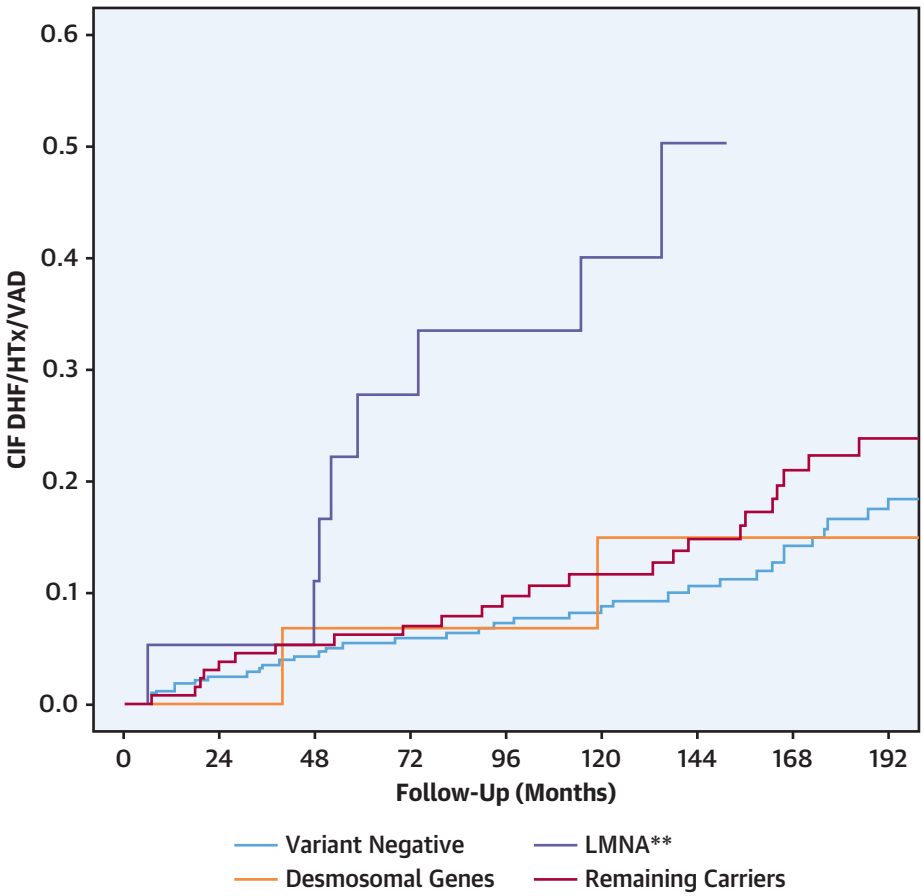
TIMELINE AND DISEASE-RELATED OUTCOMES. Genotype-phenotype interactions are ongoing issues of translational research, and the effects of mutations on the mechanisms of disease expression remain largely unknown, making longitudinal examination of a large population of genetically tested DCM patients of particular interest (26). Extreme genetic heterogeneity, low frequency, and variable penetrance prohibit robust genotype-phenotype correlation studies and, with few exceptions, genotype information does not strongly impact on clinical

TABLE 3 Outcome Measures Rates Related to Variant Status

	Variant-Negative (n = 309; 63%)	Titin (n = 54; 11%)	Lamin A/C (n = 19; 4%)	Cytoskeleton Z-Disk Genes (n = 24; 5%)	Desmosomal Genes (n = 16; 3.5%)	Sarcomeric Genes (n = 46; 9.5%)	Ion Channels Genes (n = 8; 1.5%)	Others (n = 11; 2.5%)
Age at onset, yrs	42 ± 13	42 ± 14	42 ± 14	41 ± 14	38 ± 15	39 ± 17	34 ± 15	38 ± 13
All-cause mortality*	74 (2.4)	15 (2.7)	12 (6.3)	9 (3.8)	4 (2.5)	16 (3.4)	3 (3.7)	1 (0.9)
DHF/HTx/VAD*	53 (1.7)	15 (2.7)	10 (5.3)	8 (3.3)	4 (2.5)	12 (2.6)	3 (3.7)	0 (0.0)
SCD/VT/VF*	50 (1.6)	8 (1.5)	8 (4.2)	8 (3.3)	7 (4.4)	13 (2.8)	2 (2.5)	2 (1.8)

Values are mean ± SD unless otherwise indicated. *The event-rate is expressed as n (events/100 patient-years).
DHF/HTx/VAD = heart failure death/heart transplantation/implantation of left ventricular assist device; SCD/VT/VF = sudden cardiac death/ventricular tachycardia/ventricular fibrillation.

FIGURE 3 Cumulative Incidence Curves for DHF/HTx/VAD in LMNA Carriers and Desmosomal Carriers Versus Other Patients

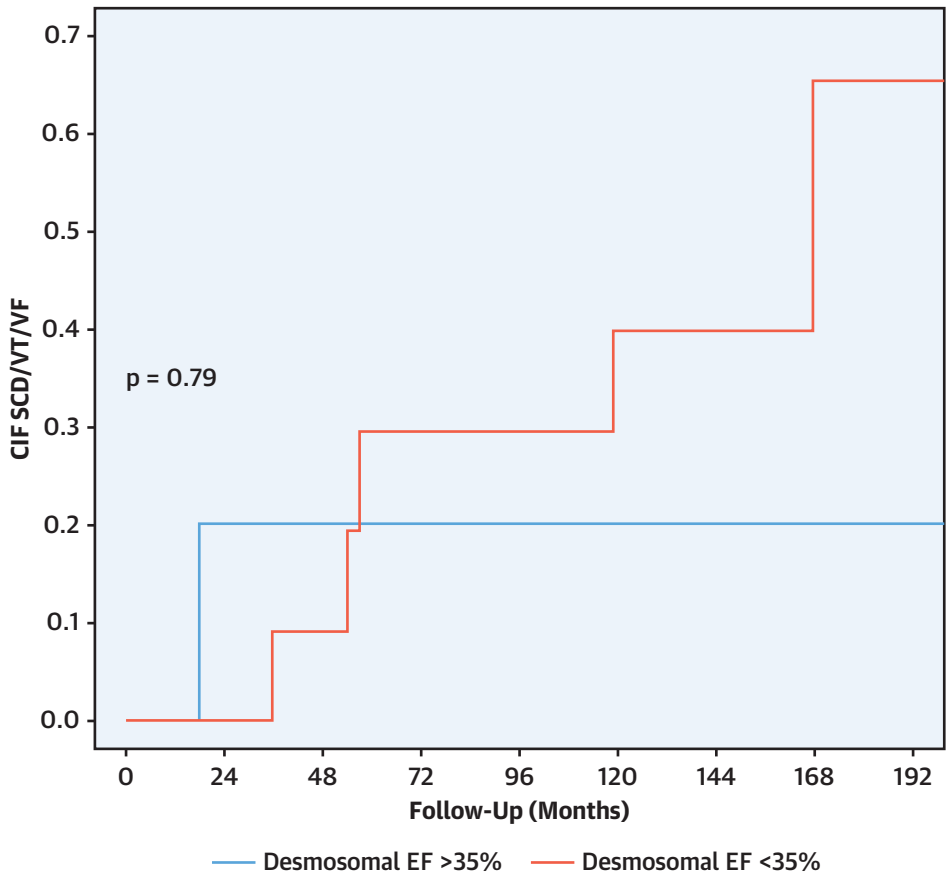


DHF/HTx/VAD in LMNA carriers **(purple line)** and desmosomal carriers **(orange line)** versus other patients. LMNA patients showed a higher risk of DHF/HTc/VAD ($p < 0.001$ for all). Abbreviations as in **Figure 2**.

management in DCM (22). Age of onset, risk of disease progression, and timing of events are examples of correlations between mutation status and phenotype expression (27). In our cohort, we observed a similar survival between variant carriers and noncarriers along a 10-year follow-up. It might be hypothesized that the effects of aging and environmental factors (e.g., comorbidities) may overcome the effect of genotype, particularly when mortality is considered overall. The strong trend toward a higher incidence of SCD/VT/VF and DHF/HTx/VAD we have observed in variant carriers (**Central Illustration, Figure 2**) supports this consideration. We also reported in the Online Appendix (Online Figure 2) the additional analysis from birth that shows higher incidence and earlier occurrence of SCD/VT/VF in variant carriers, attesting the importance of genotype information, particularly in the management of young patients with DCM.

DESMOSOMAL MUTATIONS AND RISK OF LIFE-THREATENING VENTRICULAR ARRHYTHMIAS. Although the higher rate of life-threatening arrhythmias in advanced DCM with severe HF is well-recognized and treatment algorithms are well-established, the arrhythmic risk stratification of stable patients with DCM and mild to moderate LV dysfunction represents one of the most challenging issues in the management of the disease. Conventional risk assessment fails to accurately identify "at risk" individuals. We report here that variants in desmosomal protein genes correlate with well-recognized arrhythmia syndromes independently of LVEF, as previously reported in laminopathies (5). In our study, carriers of desmosomal variants were indistinguishable from noncarriers, with the exception of a higher burden of premature ventricular complexes at 24-h ECG monitoring. We report the increased arrhythmic risk

FIGURE 4 Cumulative Incidence Curves for SCD-Related Secondary Endpoints in Patients With Desmosomal Variants According to LVEF >35% and ≤35%



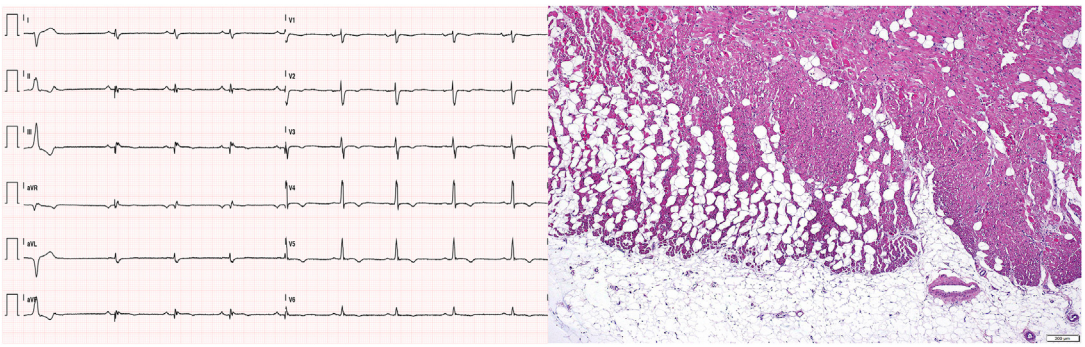
The arrhythmic risk was not related to the degree of LV dysfunction, as the likelihood of events was similar in desmosomal variant carriers with LVEF>35% versus LVEF ≤35% (p = 0.79). CIF = cumulative incidence function; LVEF = left ventricular ejection fraction; SCD/VT/VF = sudden cardiac death/sustained ventricular tachycardia/ventricular fibrillation.

in patients with a definite diagnosis of DCM carrying desmosomal variants, and this further highlights the concept of overlapping syndromes between ARVC and DCM (22). The understanding that desmosomal mutation carriers with classic DCM phenotype carry a higher risk of life-threatening arrhythmias may have important implications in the decision-making process, especially regarding the strategies to adopt for primary prevention of SCD. Although in our cohort, the limited number of variant carriers did not allow outcome correlates, it is worth mentioning that DCM genes other than *LMNA* have been recognized as "at-risk genotypes" for fatal arrhythmias, including *FLNC* and *RBM20* (6,28,29). Finally, the correlation of desmosomal variants with SCD/VT/VF was independent of left ventricular dysfunction. This represents a novel finding that mirrors the arrhythmogenic outcome of laminopathies and ARVC.

STUDY LIMITATIONS. The observational nature of the study and the small number of patients belonging to each functional gene group limit the power of the conclusions that might not be extrapolated to other populations. However, this is one of the largest cohorts of DCM patients with complete genetic characterization and prognostic information.

Comprehensive cosegregation analysis was not performed due to the limited availability of samples from relatives. In our cohort, a significant number of patients carried the variant TNNT2 R173W; this is due to both a founder effect in 1 large family (TSFDC001),

FIGURE 5 ECG and Histopathology of DCM Caused by a DSP Pathogenic Variant



(Left) The ECG of a DSP mutation (c.1816C>T p.Arg606Trp) carrier with DCM phenotype. Note the negative T waves in the anterolateral leads and the fragmented QRS that are not typical of classic DCM. **(Right)** The histopathology of patient with a DSP mutation (c.7430_7433delTGTC p.Met2477fs8aaX): section of the right ventricle showed moderate infiltration of the myocardium with adipose tissue.

and a *TNNT2* mutation hotspot as previously reported by us and others (30,31).

Data on endomyocardial biopsy, cardiac magnetic resonance, and electrophysiological testing were similarly available in only a subset of patients. This study represents the further progression of our previous work, which included 32% of the current patient population. Whereas in Spezzacatene et al. (32), we defined the specific "arrhythmogenic" DCM phenotype, here, we report the genotypes associated with high risk of arrhythmias.

Finally, our data are based on 23 disease genes selected because they represented the most frequent and well-established DCM causal genes in our population and in other study cohorts (2).

CONCLUSIONS

In line with precision medicine, our study shows that different genotypes have significantly different outcomes in DCM. In particular, desmosomal and *LMNA* variants were associated with the highest arrhythmic risk supporting the large-scale use of genetic testing to implement personalized management of DCM patients. In our study, a positive genetic test was found in 37% of DCM patients, with a higher percentage (43%) in familial DCM. Long-term survival was not significantly influenced by the mutation status, although positive carriers showed a trend toward worse prognosis in terms of HF and ventricular arrhythmias.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Mutations in desmosomal genes are typically associated with ARVC, but can also be responsible for a DCM phenotype. *LMNA* and desmosomal gene variants identify a subset of patients with DCM who are at high risk of life-threatening ventricular arrhythmias and sudden death independent of the severity of left ventricular dysfunction.

TRANSLATIONAL OUTLOOK: Future research should seek to develop algorithms for genotyping patients with DCM to guide optimum implementation of device-based therapies to prevent arrhythmic death.

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