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**Published on:** 16 Jul 2019 - European Journal of Echocardiography (Eur Heart J Cardiovasc Imaging)

**Topics:** FLNC, Cardiomyopathy and Dilated cardiomyopathy

# Dilated Cardiomyopathy and Arrhythmogenic Left Ventricular Cardiomyopathy: A Comprehensive Genotype-Imaging Phenotype Study

## Dilated Cardiomyopathy Genotype-Phenotype Associations

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**Sources of Funding** This work has been in part supported by the University College London Hospitals NIHR Biomedical Research Centre and Biomedical Research Unit at Barts Hospital. JCM and GC are supported by relevant grants from the Barts Charity.

**Disclosures** All authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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**Text word count**: 3 192

**Word count with References, Tables and Figures:** 5 116

**Abstract**

**Background:** Myocardial scar detected by cardiovascular magnetic resonance (CMR) has been associated with sudden cardiac death (SCD) in dilated cardiomyopathy (DCM). Certain genetic causes of DCM may cause a malignant arrhythmogenic phenotype. The concepts of arrhythmogenic left ventricular cardiomyopathy (ALVC) and arrhythmogenic DCM are currently ill defined. We hypothesized that a distinctive imaging phenotype defines ALVC.

**Methods and Results:** Eighty-nine patients with DCM-associated mutations (desmoplakin [*DSP*] n=25, filamin C [*FLNC*] n=7, titin n=30, lamin A/C n=12, *bcl2-associated athanogene 3* n=3, *RNA binding motif protein 20* n=3, *cardiac sodium channel* $NA_v1.5$ n=2, sarcomeric genes n=7) were comprehensively phenotyped. Clustering analysis resulted in two groups: "*DSP/FLNC* genotypes" and "non-*DSP/FLNC"*. There were no significant differences in age, sex, symptoms, baseline electrocardiography, arrhythmia burden or ventricular volumes between the two groups. Sub-epicardial LV late gadolinium enhancement with ring-like pattern (at least 3 contiguous segments in the same short axis slice) was observed in 78.1% of *DSP/FLNC* genotypes but was absent in the other DCM genotypes ($p<0.001$). LV ejection fraction and global longitudinal strain were lower in other DCM genotypes ($p=0.053$ and $p=0.015$, respectively) but LV regional wall motion abnormalities were more common in *DSP/FLNC* genotypes ($p<0.001$). *DSP/FLNC* patients with non-sustained ventricular tachycardia (NSVT) had more LV scar ($p=0.010$), whereas other DCM genotypes patients with NSVT had lower LVEF ($p=0.001$) than patients without NSVT.

**Conclusion:** *DSP/FLNC* genotypes cause more regionality in LV impairment. The most defining characteristic is a sub-epicardial ring-like scar pattern in *DSP/FLNC*, which should be considered in future diagnostic criteria for ALVC.

**Key words**: Arrhythmogenic cardiomyopathy; Dilated cardiomyopathy; Cardiac magnetic resonance; Genotype; late gadolinium enhancement

**Units and Abbreviations**

AC – Arrhythmogenic cardiomyopathy

*ACTC1* – Alpha cardiac actin

ALVC – Arrhythmogenic left ventricular cardiomyopathy

ARVC – Arrhythmogenic right ventricular cardiomyopathy

*BAG3* – Bcl2-associated athanogene 3

CMR – Cardiovascular magnetic resonance

DCM – Dilated cardiomyopathy

*DES* – Desmin

*DSP* – Desmoplakin

*FLNC* – Filamin C

ICD – Implantable cardioverter defibrillator

LBBB – Left bundble branch block

LGE – Late gadolinium enhancement

*LMNA* – Lamin A/C

*MYBPC3* – Myosin-binding protein C

*MYH7* – Beta-myosin heavy chain

NSVT – Non-sustained ventricular tachycardia

PVC – Premature ventricular contraction

RBBB – Right bundle branch block

*RBM20* – RNA binding motif protein 20

RWMA – Regional wall motion abnormalities

SCD – Sudden cardiac death

*SCN5A* – Cardiac sodium channel $NA_v1.5$

TAPSE – Tricuspid annular plane systolic excursion

*TNNI3* – Cardiac troponin I

*TPM1* – Alpha-tropomyosin

*TTN* – Titin

## Introduction

Ventricular arrhythmias and sudden cardiac death (SCD) are a significant cause of concern among patients with heart failure and dilated cardiomyopathy (DCM) (1). A left ventricular ejection fraction (LVEF) below 35% has been widely accepted as a risk marker of SCD and thus a clinical indication for prophylactic implantable cardioverter-defibrillator (ICD) (1). However, a recent clinical trial has failed to show a mortality benefit from primary prevention ICD implantation in patients with non-ischaemic DCM and the discriminative value of LVEF in isolation has been disputed (2). A more refined and personalized risk stratification taking into account the etiology of DCM could potentially identify a subset of patients at high-risk for SCD who might benefit the greatest from ICD implantation (3).

Increased use of genetic testing in clinical practice has demonstrated the magnitude of genetic heterogenicity in DCM (4). Up to 50% have a positive familial history (5) and approximately 40% have an identifiable genetic cause (6). Over 60 genes associated with familial DCM have been described (7), titin (*TTN*) being the most prevalent (20-25% of familial DCM cases), followed by lamin A/C (*LMNA*, 5-10%). Importantly, certain genes such as *LMNA*, cardiac sodium channel $NA_v1.5$ (*SCN5A*), filamin C (*FLNC*) and desmoplakin (*DSP*) may present a more malignant arrhythmogenic phenotype irrespective of the degree of LV systolic dysfunction and/or dilatation (8). This "arrhythmogenic DCM" phenotype overlaps with the current concept of arrhythmogenic cardiomyopathy (AC) (5),(9),(10) which can occur in up to one-third of DCM patients. Indeed, unlike arrhythmogenic right ventricular cardiomyopathy (ARVC)(11), left-dominant AC (ALVC) remains under-recognized due to a lack of specific diagnostic criteria (12). *DSP*(13) and *FLNC*(14) have been described as genetic causes of ALVC (9),(10). In patients initially referred for LV systolic dysfunction and/or dilatation, the

identification of specific genotypes may prompt earlier SCD risk stratification. The extent, pattern and location of myocardial scar detected in cardiovascular magnetic resonance (CMR) have also been associated with SCD in DCM (15).

We hypothesize that there is a characteristic ALVC imaging phenotype that is distinct from other DCM-associated genes and thus explore the comprehensive phenotype of DCM/ALVC related genes - structure/function, electrical and tissue characterization - to uncover specific genotype-phenotype correlations that could form the basis of future diagnostic criteria and therapeutic approaches.

## Methods

*Study population and data collection*

An observational, retrospective, single-center study of patients from the Inherited Cardiovascular Disease Department at Barts Heart Centre, London, UK. All patients gave written informed consent in accordance with the protocol approved by the regional ethics committee (15/LO/0549). Ethical approval for the analysis of these imaging data also falls within the scope of an institutional audit (5298). Institutional genetic databases were interrogated for variants associated with DCM. We included consecutive carriers of likely pathogenic/pathogenic variants associated with DCM, who also underwent CMR between May 2011 and May 2018. Patient selection was performed by one author (L.L.), blinded to the imaging results.

We excluded from our analysis patients with the following: (1) age under 18 years, (2) non-pathogenic variants or variants of unknown clinical significance, (3) more than one likely pathogenic/pathogenic variant, (4) coronary artery disease, (5) significant primary valvular disease, (6) uncontrolled hypertension and/or toxin exposure sufficient to cause a myocardial abnormality and/or (7) congenital heart disease.

*Genetic analysis*

Patients with likely pathogenic/pathogenic variants in the following genes were included (Supplementary Table 1): *DSP*, *FLNC*, *LMNA*, *SCN5A, LMNA*, bcl2-associated athanogene 3 (*BAG3*), RNA binding motif protein 20 (*RBM20*), *TTN* and sarcomeric genes (including alpha cardiac actin [*ACTC1*], myosin-binding protein C [*MYBPC3*], beta-myosin heavy chain [*MYH7*], cardiac troponin I [*TNNI3*] and alpha-tropomyosin [*TPM1*]) (13),(14),(7).

*Clinical analysis*

Clinical data at the time of CMR was collected by cardiologists blinded to both CMR and genetic status (J.A., R.E.).

All patients included met the clinical criteria for early/pre-clinical or clinical DCM (5) – LV cavity dilatation and/or impaired ejection fraction with reference to age- and gender-adjusted CMR nomograms(16), including hypokinetic non-dilated cardiomyopathy and isolated LV dilatation, in the absence of significant coronary artery disease, uncontrolled hypertension, significant primary valvular disease, toxin exposure and congenital heart disease sufficient to cause the observed myocardial abnormality (17).

All patients were also post-hoc assessed for the presence of ARVC diagnostic criteria (2010 revised task force criteria(11)) and for the presence of previously described features of ALVC(12): (1) unexplained arrhythmia of LV origin (polymorphic or right bundle branch block [RBBB] morphology), (2) (infero)lateral T-wave inversion, (3) LV dilatation / systolic dysfunction with arrhythmic presentation.

*Electrocardiogram and Holter*

We recorded ECG abnormalities according to the ARVC revised task force criteria 2010(11). All patients underwent 24-hour Holter monitoring at least once a year as part of routine assessment. Holter data after the CMR was evaluated. Ventricular tachycardia (VT) was classified as non-sustained (NSVT) if duration was less than 30 seconds. VT morphology was recorded: left (LBBB) or right (RBBB) bundle branch block. Presence and morphology of frequent premature ventricular contractions (PVCs >1,000 per 24 hours) or frequent couplets (≥50 per 24 hours) on Holter monitoring were also noted. Presence of ICD (at the time [n=3] or after CMR) and clinical indication for ICD (primary or secondary prevention) were recorded. We also determined the occurrence of major arrhythmic events after CMR, which included appropriate ICD interventions (appropriate shock and/or anti-tachycardia pacing), sustained VT, ventricular fibrillation (VF) and aborted SCD, noting age at first arrhythmic event.

*Cardiovascular magnetic resonance*

All participants underwent CMR at 1.5 Tesla or 3 Tesla (Aera and Avanto 1.5T; Prisma 3T scanner; Siemens Healthcare, Erlangen, Germany). If more than one CMR was performed, only the most recent one was considered for analysis. The acquisition protocol is described in detail in the Supplementary Material. All images were analyzed using CVI42 software (Circle Cardiovascular Imaging Inc., Calgary, Canada). Measurements were performed by two experienced cardiologists blinded to both clinical and genetic data (E.N., S.M.F.). LV LGE (scar) quantification was performed in the short-axis slices using manually drawn endocardial and epicardial borders and a semi-automated 5 SD approach with minimal manual adjustment, and expressed in grams and as a percentage of total LV mass. Presence or absence of RV LGE was also noted. LGE pattern was defined as ring-like if there were at least 3 contiguous segments with sub-epicardial LGE in the same

slice (although typically the ring was more complete than this). Endocardial and epicardial borders were manually drawn in the mid short axis slice (20% offset) and mean T1 values were obtained for the slice. Global radial, longitudinal and circumferential 2D strain values were obtained using feature tracking analysis.

*Statistical analysis*

A detailed description of the statistical analysis is provided in the Supplementary Material. Briefly, all subjects were clustered post-hoc according to their genotype and imaging phenotype, the latter including indexed LV end-diastolic volume, LVEF, percentage of LGE in total LV mass (%LGE) and presence/absence of RV LGE. A two-step cluster analysis was performed with two predefined clusters and using a log-likelihood statistic as a distance measurement. Grouped/clustered genotypes were then compared using Students' *t*-test or Mann-Whitney *U* test (parametric and nonparametric data respectively); categorical variables were compared using Fisher's exact test. Differences between individual genotypes were assessed using one-way ANOVA for parametric data or Kruskal–Wallis test for nonparametric data; categorical variables were compared using Chi-square test. Significant effects were further evaluated with post-hoc pairwise comparisons with Bonferroni adjustment. Two-sided $p$-values $<0.05$ were considered significant.

## Results

*Study population*

We included 89 patients (76 probands, 13 affected relatives). Mean age was 45.9 ± 1.5 years, 51 (57.3%) were males. Pathogenic variants included 30 *TTN*, 25 *DSP*, 12 *LMNA*, 7 *FLNC*, 3 *BAG3*, 3 *RBM20*, 3 *MYH7*, 2 *SCN5A* and 7 other sarcomeric genotypes (1 *TNNI3*, 1 *TPM1*, 1 *ACTC1*, 1 *MYBPC3*, see Supplementary Table 1). Two clusters were derived, one with *DSP* and *FLNC* genotypes, the other with the remaining DCM genotypes. Demographic characteristics are summarized in Table 1 (clustered genotypes) and Supplementary Table 3 (individual genotypes). Individual and aggregated genotypes were similar in terms of age, sex, ethnicity and proband/relative ratios.

*Symptoms and Electrocardiography*

There were no significant differences in symptoms, medications or ICD implantation between groups or among individual genotypes (Table 1 and Supplementary Table 3, respectively).

Patients were followed for 32.4±17.4 months. There was a trend for premature ventricular contractions and VTs to be more frequently polymorphic in the *DSP/FLNC* group (37.5 vs 19.3% in the other DCM genotypes, p=0.079). No other differences were seen regarding baseline ECG changes / arrhythmias between aggregated (Table 2) or individual genotypes (Supplementary Table 4).

*Cardiovascular Magnetic Resonance*

All images were analyzable. Imaging phenotypes are summarized in Table 3, Supplementary Table 5 (for aggregated and individual genotypes, respectively) and in

Figures 1 to 4. LV ejection fraction was tendentially lower in the other DCM genotypes (44.0±11.4% vs 49.0±12.2% in *DSP/FLNC*, p=0.053). LV global longitudinal strain (GLS, absolute value) and tricuspid annular plane systolic excursion (TAPSE) were significantly lower in the other DCM genotypes (p=0.015 and p=0.003, respectively, Table 3) with LV and RV regional wall motion abnormalities (RWMA) being more common in *DSP/FLNC* (p<0.001 for both, Table 3). LV and RV myocardial fat infiltration were solely seen in *DSP/FLNC* (31.6% and 15.8%, respectively, Table 3).

*LGE in DCM genotypes*. LGE was significantly more common in *DSP/FLNC* in both LV and RV (LV 90.6 vs 68.4%, p=0.020; RV 21.9 vs 1.8%, p=0.003, Figure 1, Supplementary Table 6). Within other DCM genotypes, scar was not different between *LMNA* and the other non-*LMNA* genotypes (2.2% (0 – 8.6) vs 3.1% (0.5 – 12.4), p=0.253). Scar pattern was also different. In *DSP/FLNC* scar was typically found in the basal lateral segments, characteristically subepicardial (87.5 vs 12.3% in other DCM genotypes, p<0.001, Figure 2) with a ring-like pattern in 78.1% (84.0% *DSP*, 57.1% *FLNC*) which was not seen in any of the other DCMs (p<0.001). Scar distribution is further detailed for individual genotypes in Figures 3 and 4. *DSP/FLNC* genotypes without scar had no LV RWMAs and overall normal ejection fraction (minimum LVEF was 52.0%), whereas those with scar had motion abnormalities in 51.7% (vs 0%, p=0.229) and significantly reduced LV ejection fraction (47.4±11.0 vs 64.3±15.7%, p=0.020). In the other DCM genotypes impairment was frequently present without scar and scar presence did not significantly change EF (mean LVEF 42.6±11.4 with vs 46.8±11.2% without scar, p=0.198).

*Arrhythmias and CMR characteristics*. *DSP/FLNC* with NSVT had more extensive LV scar (%LV mass median 19.5 (13.6–33.9) vs 11.3 (6.3–16.8), p=0.010) and a trend for lower LVEF (44.7±10.9% vs 52.9±12.3, p=0.056) than patients without NSVT. Within other DCM genotypes, scar was not different between patients with and without NSVT (%LV mass median 2.2 (0 – 3.9) vs 0.6 (0 – 2.7), p=0.310); in contrast, those with NSVT had lower LVEF (37.8±11.4% vs 47.6±9.9% in patients without NSVT, p=0.001).

*ARVC and ALVC criteria*. ARVC criteria were met in 5 out of 89 patients, all within *DSP/FLNC* genotypes (specifically all patients with *DSP* mutation, no ARVC criteria were found among DCM genotypes, p=0.005, Table 3). The prevalence of typical ALVC features (arrhythmia of LV origin, inferolateral T-wave inversion, LV dilatation/systolic dysfunction with arrhythmic presentation) was not significantly different between *DSP/FLNC* and the other DCM genotypes (53.1 vs 38.6%, respectively, p=0.266). Adding the ALVC features to the presence of the aforementioned ring-like LGE to *DSP/FLNC* would not significantly increase the percentage of ALVCs identified (from 78.1 to 90.6%, p=0.125), but there would be a significant increase in the other DCM genotypes with ALVC features (from 0 to 38.6%, p<0.001).

## Discussion

This is the first study systematically comparing clinical and imaging phenotypes in patients with pathogenic DCM genotypes. The main findings of this study are three-fold. First, by performing a clustering analysis of genotype and CMR data, we found a characteristic subepicardial, ring-like scar pattern associated with *DSP* and *FLNC* genotypes which was not present in the other DCM genotypes; this likely corresponds to a distinct entity – ALVC. Second, LV impairment in *DSP/FLNC* is scar-related whereas

in the other DCM genotypes, LV impairment can occur without scar and is relatively scar independent. Third, while the other DCM genotypes have more impaired LVEF and GLS (despite same degree of LV dilatation), *DSP/FLNC* have more regionality in LV impairment. The acknowledgement of the aforementioned CMR patterns in patients being investigated for DCM (LV systolic dysfunction and/or dilatation) should thus raise a suspicion of ALVC-associated genotypes.

*Imaging phenotype in Desmoplakin and Filamin C*

*DSP/FLNC* have been more consistently described as ALVC-associated genes in the literature (9)(10)(13)(14). Recognition of ALVC however poses a diagnostic and nomenclature challenge due to the absence of defining criteria. Our finding of a sub-epicardial "ring of fibrosis" represented a very specific imaging hallmark, which was present only in *DSP* and *FLNC* and not in any other DCM genotypes. Moreover, its high prevalence in ALVC genotypes (*DSP/FLNC*, approximately 80%) makes it a feature that should be actively looked for in patients with suspected ALVC. Even in cases with clear ARVC presentation (such as RV dyskinesia or RV aneurysms), the detection of left sided involvement is limited if only function is assessed – half of the ALVCs with LV LGE had no wall motion abnormalities. Scar is a key aspect. Not only causes but also precedes wall motion abnormalities. LV systolic function deteriorates in the presence of LV fibrosis (being preserved in ALVC genotypes without LGE). In contrast, dysfunction in DCM genotypes is primarily non-scar related.

Although our findings are unique in comparing imaging findings across DCM genotypes, a similar LGE pattern has been previously noted in AC. Sen-Chowdhry et al.(18) assessed 168 ARVC patients with LV involvement in CMR and LV LGE was solely sub-epicardial or both sub-epicardial and mid-myocardial in 128 patients. In another study by the same

group(12), 40 contrast CMR scans were performed in patients with suspected or confirmed ALVC – all patients had LV LGE, which was sub-epicardial in 30% and circumferential in 20%; 15 patients had causative mutations in desmosomal genes. However, specific genotype-phenotype correlations were not addressed in both studies. Interestingly, a high proportion of LV fibrosis has also been previously shown in 29 of 49 patients with *FLNC* mutation(10); the LGE pattern was mainly sub-epicardial in these cases. We found a higher prevalence of sub-epicardial fibrosis (in 9 out of 10 patients) and characteristic ring-like LGE pattern (in 8 out of 10) in patients with confirmed pathogenic *DSP* and *FLNC* mutations.

Other diseases may present a similar LGE pattern, including myocarditis, Duchenne and Becker muscular dystrophies. Muscular dystrophies however would be discernable by their systemic presentation. Myocarditis on the other hand might constitute a challenge as this often goes unnoticed. Referrals for LV dilatation / systolic dysfunction are very common in clinical practice. Using previously described ALVC features (such as arrhythmia of LV origin or inferolateral T-wave inversion)(12) alone may be insufficient to discriminate between ALVC and DCM genotypes. In contrast, using the highly characteristic ring-like scar we described it is reasonable to include *DSP/FLNC* genes in the differentials and thus discrimination between ALVC and DCM can be improved.

*Imaging phenotype in other DCM genotypes*

Overall, patients with other DCM-associated mutations had smaller amounts of LV scar but more biventricular systolic dysfunction (as assessed by LVEF, GLS and TAPSE); the presence of scar did not significantly impact on LVEF, suggesting that for the majority of its causing genotypes, DCM is a disease where LV impairment is myocyte dysfunction rather than scar related.

No specific CMR trait was helpful in the distinction between individual DCM genotypes. Association between *BAG3* and DCM has been ascertained(19), but no studies specifically addressed CMR imaging phenotypes. In a large genotype-phenotype study of DCM patients, there were no significant differences between *TTN* positive and *TTN* negative patients in terms of LV dilatation, LV systolic dysfunction or LGE amount/pattern(20). A recent study has suggested that *RBM20* is related to an increased rate of sustained ventricular arrhythmias in comparison with *TTN* (44 vs 5%)(21), despite similar degrees of LV dilatation and LV systolic dysfunction in both groups (but CMR patterns were out of the scope of this study). We found no significant difference in the CMR traits between *RBM20* and *TTN* in our cohort. In a study of 17 patients with DCM and *LMNA* mutation who underwent CMR, 15 subjects showed LV LGE which was mid-myocardial in most cases(22). Likewise, we found a similar distribution of LV scar in two thirds of *LMNA* patients. However, we did not find any specific imaging pattern that could allow us discriminating between *LMNA* and other DCM genotypes. In contrast, electrical abnormalities such as atrioventricular conduction disturbances and atrial fibrillation have recently been found to have a high prevalence in *LMNA* genotypes (23). To the best of our knowledge, there are no dedicated studies investigating the imaging phenotype of other sarcomeric genes in DCM besides *TTN*. We showed that LGE in these patients was found in the midwall and mainly in the basal-to-mid septum, which is non-specific for distinction from other non-*DSP/FLNC* DCM-associated genotypes. Van Hoorn et al. assessed 40 *SCN5A* patients with Brugada syndrome(24); CMR was performed in all of them – fatty infiltration was found in 1 patient (2.5%) and fibrosis was seen in 3 patients (7.5%), the location of which was not specified. Further characterization of scar localization among DCM patients with *SCN5A* variants is warranted.

*Clinical phenotypes and diagnostic work-up*

Detecting more arrhythmogenic DCM phenotypes can have important clinical implications in terms of risk stratification, familial evaluation and management. LVEF alone might be insufficient to identify patients who are at risk(2),(25). Genotyping has already been proven to be useful in this regard, particularly for lamin A/C(26),(1). Scar imaging has also been shown to be a promising risk stratification marker(27).

While the finding of a ring-like scar has diagnostic value in *DSP/FLNC* genotypes, the impact of specific DCM genotypes and corresponding imaging features on outcomes deserves further investigation. The total prevalence of ventricular arrhythmias was similar between the two genotype clusters in this study. However and importantly, extensive LV scar was associated with NSVT in *DSP* and *FLNC* genotypes but not in other DCM genotypes, where the overall extent of scar was low. On the other hand, lower LVEF was associated with NSVT in both genotype groups, but LVEF was significantly higher in the *DSP/FLNC* cluster. The combination of these imaging features likely accounts for the similar number of arrhythmic events.

Nonetheless, in light of the heterogeneity of non-*DSP/FLNC* genotypes, conclusions regarding risk of ventricular arrhythmias in individual genotypes (such as *LMNA*) cannot be formally drawn. In fact, the association between LV scar in patients with *LMNA* and ventricular arrhythmias has also been described (28). A recent study(15) also reported that concomitant LGE in the septum and free-wall (possible reflecting ring-like scar) accounted for the greatest risk of SCD in DCM patients. Moreover, when the scar was subepicardial or had multiple patterns, the risk of SCD was also higher. Our data strongly suggests that these imaging differences could be accounted for by a distinct genetic background.

*Limitations*

ARVC was defined according to the Task Force Criteria 2010. Other consensus on AC have been published since then (29), but these are not widely used and do not provide formal diagnostic criteria. Furthermore, most of the additional imaging parameters described for the diagnosis of AC are mainly RV-focused, highlighting the gap of knowledge in ALVC.

We used stringent inclusion criteria for patients with both likely pathogenic/pathogenic variants and a CMR. Advanced lamin A/C patients may have been under-represented as they typically receive ICDs before CMR scanning is done. Fat suppression and mapping sequences were not performed in all patients and their value in the identification of specific DCM genotypes has not been thoroughly investigated; this, however, reflects standard CMR acquisition protocols according to the referral or specific clinical question.;

**Conclusions**

Advances in two key areas of cardiomyopathy are increasing. Firstly, the increased availability of genetic testing, and secondly *in-vivo* myocardial tissue characterization by CMR, particularly LGE. The harmonization of these viewpoints into an integrated understanding based on concurrent genotype-phenotype appreciation promises new specific management strategies and therapeutic approaches. Continued efforts are needed to develop and validate prognostic models combining imaging and phenotype to identify high-risk subgroups who might benefit from ICD implantation.

We showed that when analyzing a thoroughly genotyped and phenotyped cohort of DCM patients and defining two groups through a clustering analysis according to genotype and imaging characteristics, *DSP/FLNC* genotypes (consistently described as ALVC-

associated genes in the literature) caused a more heterogeneous phenotype with more regionality in LV impairment. The most characteristic difference is that *DSP/FLNC* patients frequently had a ring-like late gadolinium enhancement pattern that did not occur in other genotypes. We propose that this new defining feature should be considered in future diagnostic criteria for ALVC, while deserving further testing as a risk factor for sudden cardiac death.

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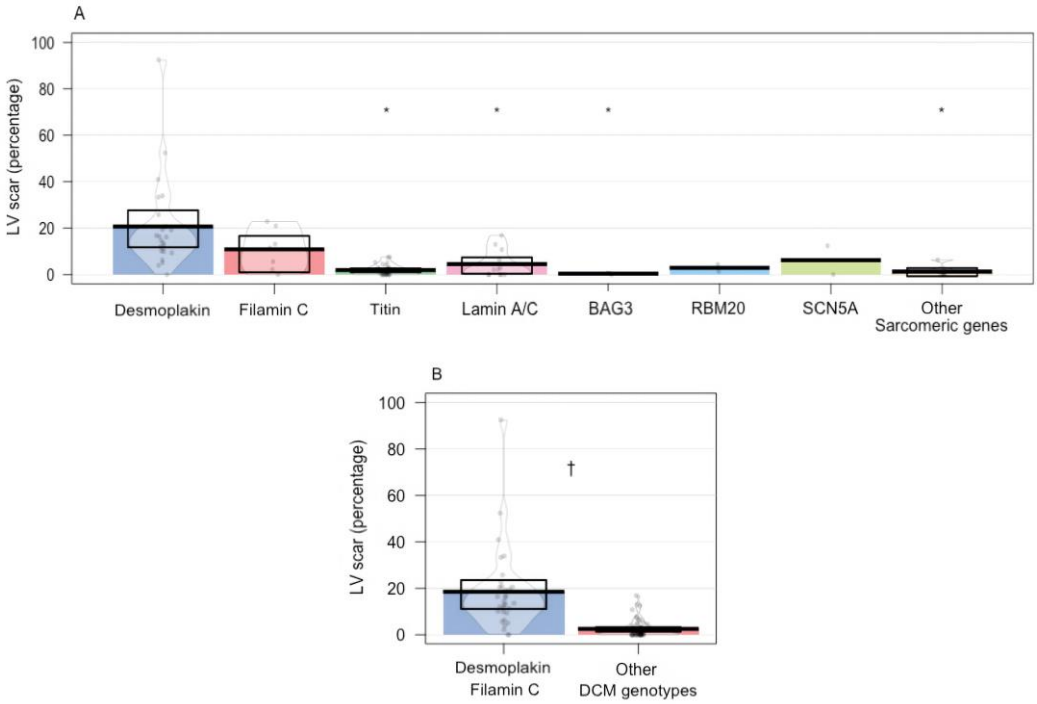
## Figures

**Figure 1.** Box plots and confidence intervals comparing the percentage of left ventricular scar among individual genotypes (A) and between grouped genotypes (B).

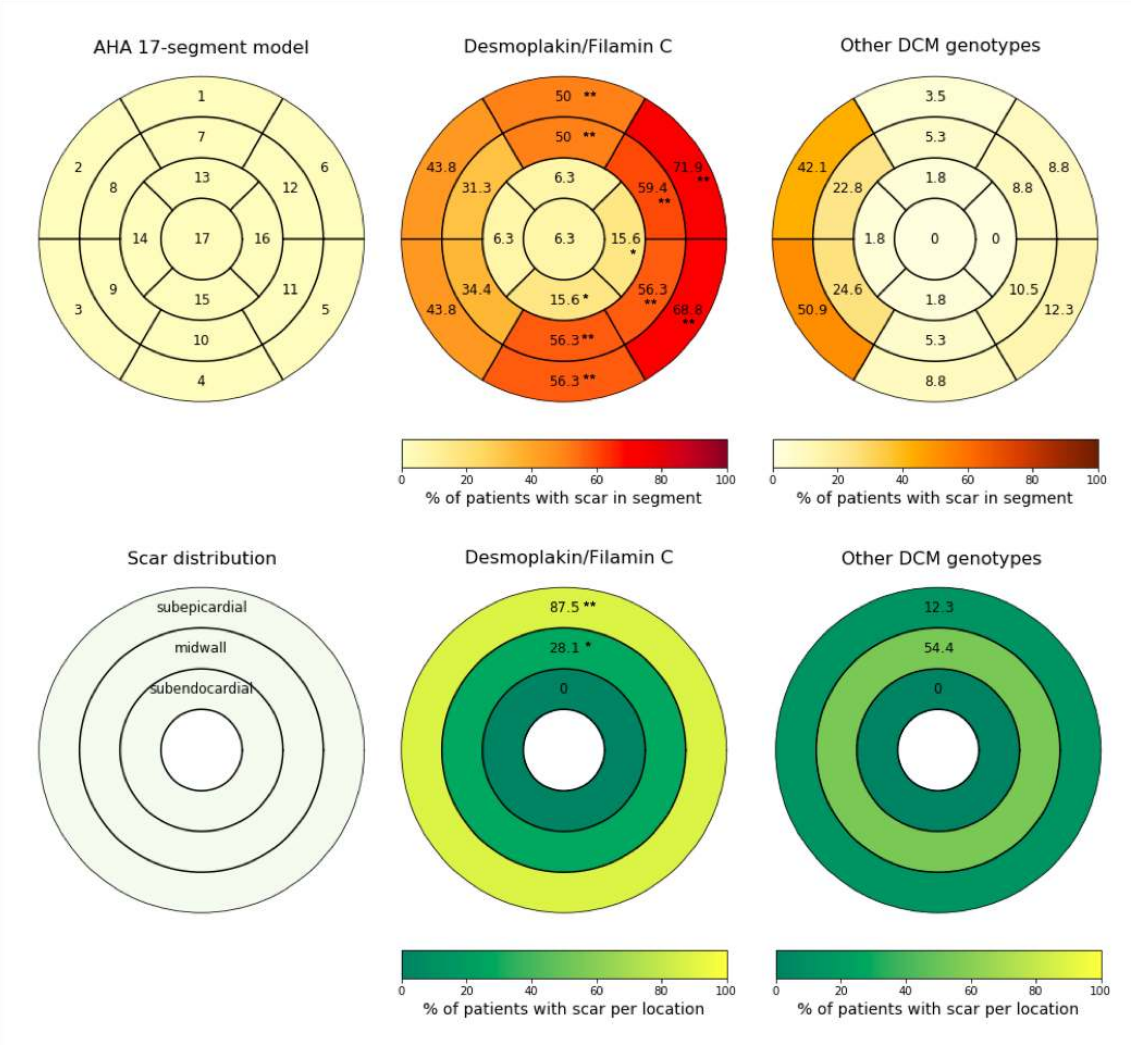



Legend: *BAG3*, bcl2-associated athanogene 3; DCM, dilated cardiomyopathy; *RBM20*, RNA binding motif protein 20; SCN5A, cardiac sodium channel $NA_v1.5$.

* *P*-value <0.002 vs Desmoplakin.

† *P*-value <0.05.

**Figure 2**. Distribution of left ventricular scar in myocardial layers and in bull's eye view of the 17-segment AHA according to aggregated genotypes.

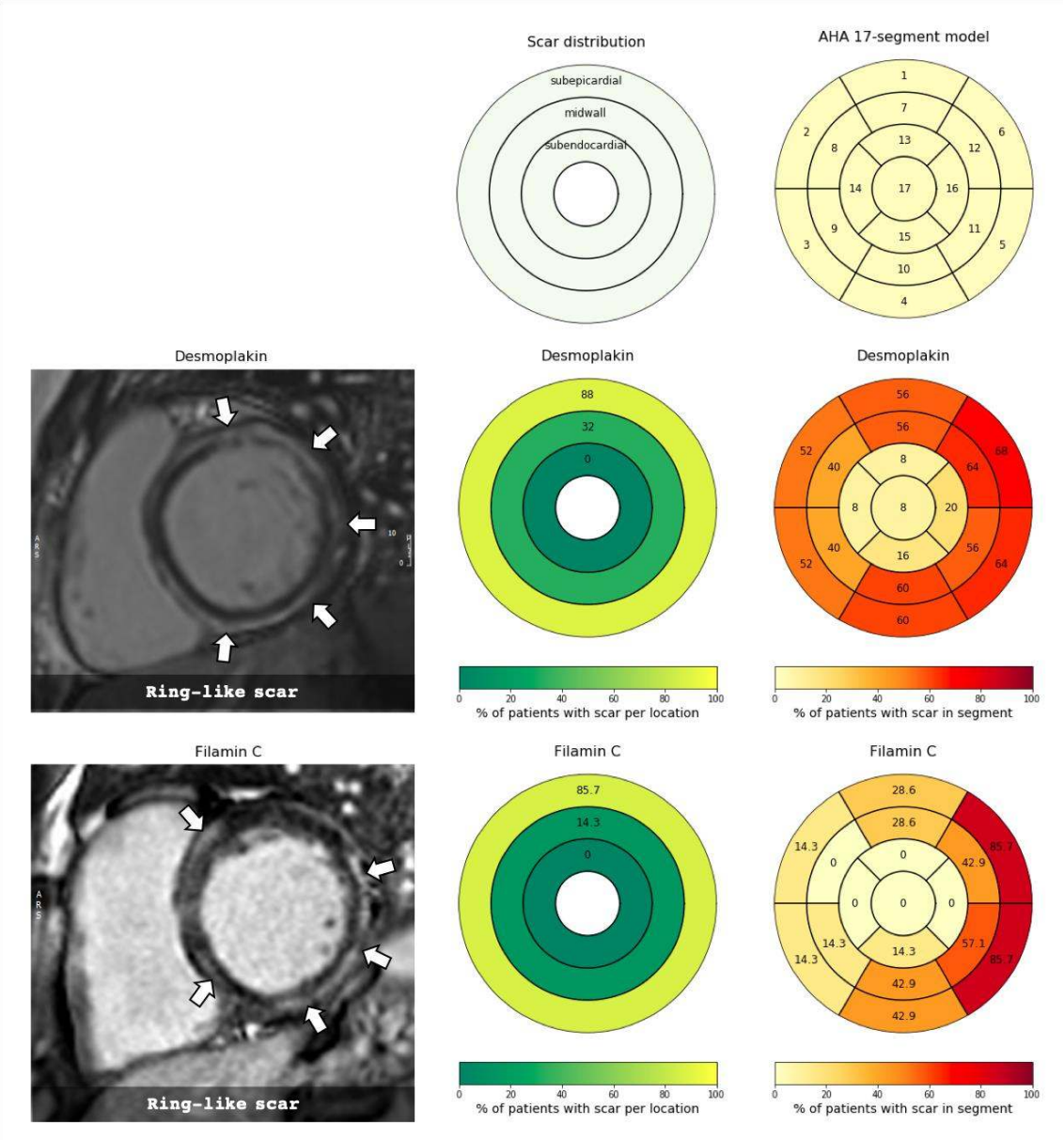



Legend: AHA, American Heart Association; DCM, dilated cardiomyopathy.

* *P*-value < 0.05 vs corresponding segment/myocardial layer in other DCM genotypes.

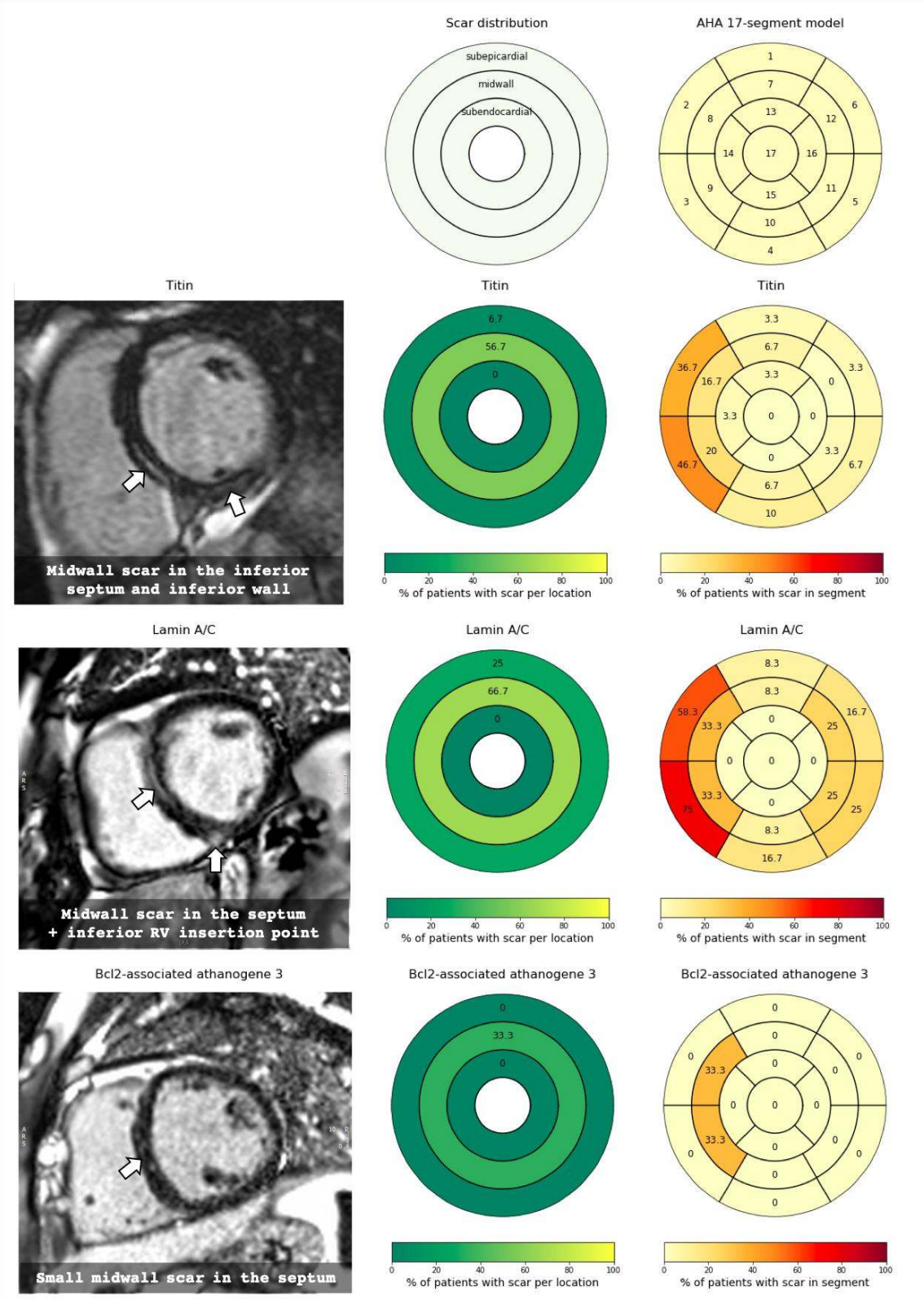
** *P*-value < 0.001 vs corresponding segment/myocardial layer in other DCM genotypes.

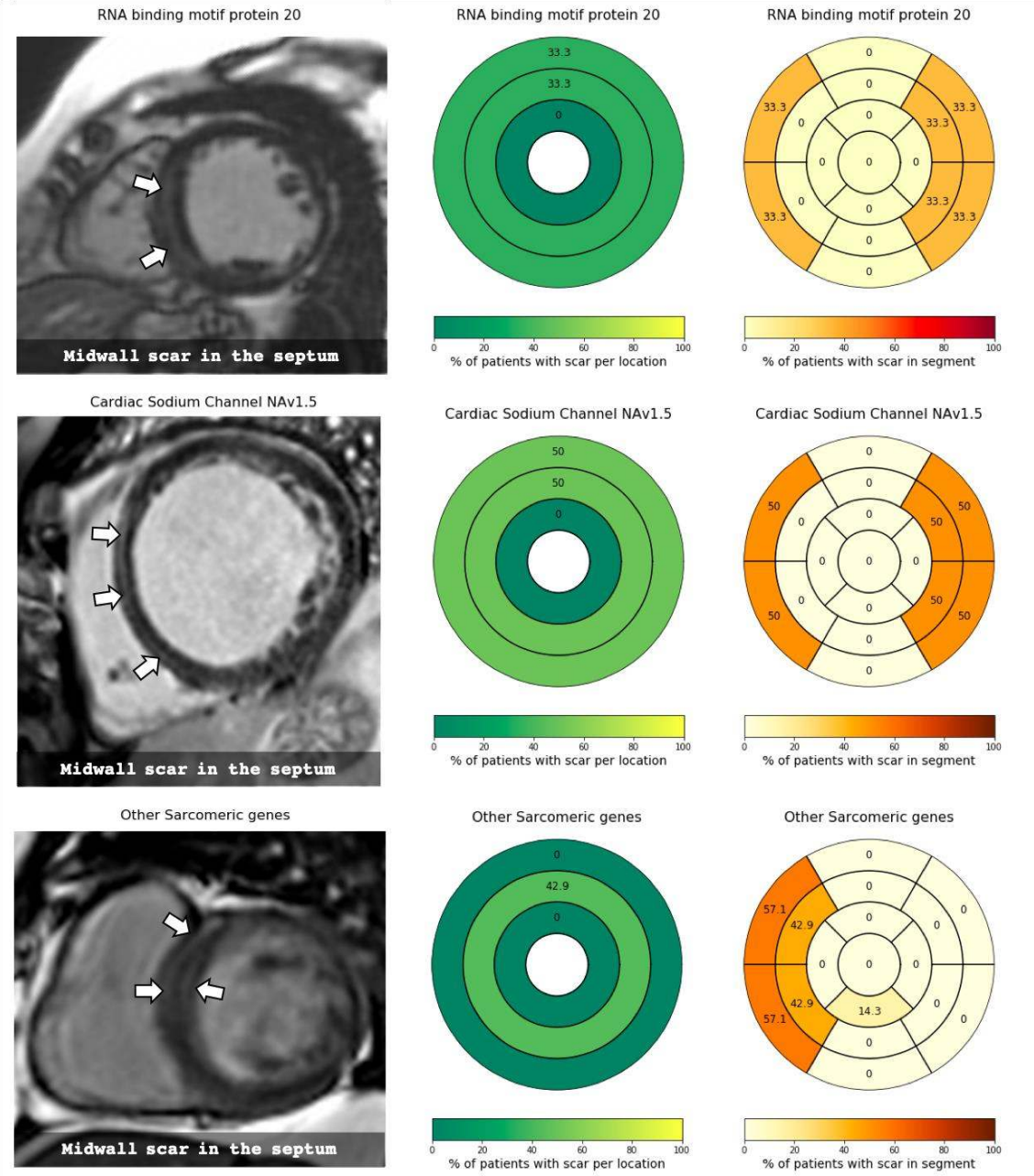
**Figure 3**. Distribution of left ventricular scar in myocardial layers and in bull's eye view of the 17-segment AHA for desmoplakin/filamin C genotypes.




Legend: AHA, American Heart Association. White arrows indicate late gadolinium enhancement.

**Figure 4**. Distribution of left ventricular scar in myocardial layers and in bull's eye view of the 17-segment AHA for other DCM genotypes.






Legend: AHA, American Heart Association; DCM, dilated cardiomyopathy; RV, right ventricle. White arrows indicate late gadolinium enhancement.

## Tables

**Table 1**. Demographic and clinical characteristics of the study population according to the genotype group.

| | **Total population (n=89)** | ***DSP/FLNC* (n=32)** | **Other DCM genotypes (n=57)** | ***P value**** |
|---|---|---|---|---|
| **Demographic characteristics** | | | | |
| Male | 51 (57.3) | 15 (46.9) | 36 (63.2) | 0.181 |
| Age at diagnosis, years | 42.2 ± 13.6 | 42.3 ± 14.5 | 42.1 ± 13.2 | 0.941 |
| Caucasian | 81 (91.0) | 30 (93.8) | 51 (89.5) | 0.706 |
| Proband | 76 (85.4) | 25 (78.1) | 51 (89.5) | 0.211 |
| **Clinical characteristics** | | | | |
| Symptoms | 38 (42.7) | 11 (34.4) | 27 (47.4) | 0.270 |
| Family history of DCM | 43 (48.3) | 12 (37.5) | 31 (54.4) | 0.184 |
| Family history of AC | 12 (13.6) | 11 (34.4) | 1 (1.8) | **<0.001** |
| Family history of SCD / VT / VF | 35 (39.3) | 14 (43.8) | 21 (36.8) | 0.652 |
| ICD | 32 (36.0) | 15 (46.9) | 17 (29.8) | 0.116 |
| Primary prevention | 24 (75.0) | 11 (73.3) | 13 (76.5) | 1.000 |
| Medication | | | | |
| Beta-blocker | 69 (77.5) | 24 (75.0) | 45 (78.9) | 0.792 |
| Anti-arrhythmic | 8 (9.0) | 1 (3.1) | 7 (12.3) | 0.250 |
| ACEi / ARB | 68 (76.4) | 23 (71.9) | 45 (78.9) | 0.450 |

ACEi, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blockers; DCM, dilated cardiomyopathy; *DSP*, desmoplakin; *FLNC*, filamin C; ICD, implantable cardioverter-defibrillator; SCD, sudden cardiac death; VF, ventricular fibrillation; VT, ventricular tachycardia.

Discrete variables are presented as n (%) and continuous variables as mean ± SD.

* *P*-value for DSP/FLNC vs. other DCM genotypes. *P*-values < 0.05 are expressed in bold.

**Table 2**. Arrhythmic events and electrocardiographic characteristics of the study population according to the genotype group.

| | **Total population (n=89)** | ***DSP/FLNC* (n=32)** | **Other DCM genotypes (n=57)** | ***P value**** |
|---|---|---|---|---|
| TWI V2-V3 | 6 (7.1) | 4 (12.5) | 2 (3.8) | 0.192 |
| TWI V4-V6 | 12 (14.1) | 6 (18.8) | 6 (11.3) | 0.355 |
| LBBB | 2 (2.2) | 0 | 2 (3.5) | 0.534 |
| RBBB | 2 (2.2) | 1 (3.1) | 1 (1.8) | 1.000 |
| ≥1,000 PVCs / 24h | 37 (41.6) | 15 (46.9) | 22 (38.6) | 0.505 |
| ≥50 couplets / 24h | 13 (14.6) | 5 (15.6) | 8 (14.0) | 1.000 |
| Non-sustained VT | 36 (40.4) | 15 (46.9) | 21 (36.8) | 0.377 |
| Sustained VT | 2 (2.2) | 1 (3.1) | 1 (1.8) | 1.000 |
| PVC / VT morphology | | | | |
| LBBB-like | 7 (7.9) | 2 (6.3) | 5 (8.8) | 1.000 |
| RBBB-like | 9 (10.1) | 2 (6.3) | 7 (12.3) | 0.480 |
| Polymorphic | 23 (25.8) | 12 (37.5) | 11 (19.3) | 0.079 |
| Aborted cardiac arrest | 5 (5.6) | 2 (6.3) | 3 (5.3) | 1.000 |
| ICD appropriate interventions | 2 (6.3) | 0 | 2 (11.8) | 0.486 |
| Major arrhythmias | 10 (11.2) | 6 (18.8) | 4 (7.0) | 0.158 |
| Age at first arrhythmic event, years | 48.0 (21.0) | 39.0 (20.0) | 49.0 (11.0) | 0.189 |

DCM, dilated cardiomyopathy; *DSP*, desmoplakin; *FLNC*, filamin C; ICD, implantable cardioverter-defibrillator; LBBB, left bundle branch block; PVCs, premature ventricular contractions; RBBB, right bundle branch block; TWI, T wave inversion; VF, ventricular fibrillation; VT, ventricular tachycardia.

Discrete variables are presented as n (%) and continuous variables as median (interquartile range).

* *P*-value for *DSP/FLNC* vs. other DCM genotypes.

**Table 3**. Imaging phenotypes in cardiac magnetic resonance according to the genotype group.

| | **Total population (n=89)** | ***DSP/FLNC* (n=32)** | **Other DCM genotypes (n=57)** | ***P value**** |
|---|---|---|---|---|
| LV EDVi, ml/m$^2$ | 100.0 (29.0) | 101.0 (31.5) | 99.0 (32.3) | 0.925 |
| LV ESVi, ml/m$^2$ | 52.0 (29.0) | 49.0 (35.5) | 53.0 (24.0) | 0.243 |
| LV CI, L/min | 3.1±0.7 | 3.1±0.7 | 3.1±0.7 | 0.937 |
| LV EF, % | 45.8±11.9 | 49.0±12.2 | 44.0±11.4 | 0.053 |
| LV RWMA | 18 (20.2) | 15 (46.9) | 3 (5.3) | **<0.001** |
| MAPSE, mm | 13.4±3.8 | 13.1±3.5 | 13.5±3.9 | 0.639 |
| LV MI, g/m$^2$ | 64.2±16.9 | 65.4±18.0 | 63.6±16.4 | 0.633 |
| MWT, mm | 9.0 (2.0) | 8.6 (1.3) | 9.0 (2.0) | **0.037** |
| RV EDVi, ml/m$^2$ | 82.8 (31.7) | 91.5 (34.0) | 79.9 (32.0) | 0.068 |
| RV ESVi, ml/m$^2$ | 37.0 (21.2) | 42.1 (25.0) | 36.0 (21.5) | 0.159 |
| RV CI, L/min | 3.0±0.8 | 3.0±0.8 | 3.0±0.8 | 0.834 |
| RV EF, % | 53.0 (12.0) | 53.0 (15.0) | 53.2 (10.9) | 0.800 |
| RV RWMA | 10 (11.2) | 10 (31.3) | 0 | **<0.001** |
| TAPSE, mm | 20.2±4.9 | 22.2±4.5 | 19.0±4.8 | **0.003** |
| LA area, cm$^2$ | 25.0 (8.0) | 24.9 (9.4) | 25.0 (7.4) | 0.421 |

| | | | | |
|---|---|---|---|---|
| RA area, $cm^2$ | 23.0 (9.0) | 23.0 (8.2) | 23.2 (9.0) | 0.905 |
| LV fat infiltration | 6/25 (24.0) | 6/19 (31.6) | 0/6 | 0.278 |
| RV fat infiltration | 3/25 (12.0) | 3/19 (15.8) | 0/6 | 0.554 |
| ARVC criteria | 5 (5.6) | 5 (15.6) | 0 | **0.005** |
| ALVC features | 39 (43.8) | 17 (53.1) | 22 (38.6) | 0.266 |
| Native T1 mapping, ms | 1025.6 (64.0) | 1026.9 (28.2) | 1023.0 (73.0) | 0.990 |
| LV GRS, % | 30.8 (19.1) | 29.2 (22.6) | 31.1 (13.7) | 0.905 |
| LV GCS, % | -14.2 (6.2) | -15.2 (6.2) | -13.7 (4.5) | 0.110 |
| LV GLS, % | -11.9 (4.7) | -14.1 (6.0) | -11.2 (3.8) | **0.015** |

CI, cardiac index; DCM, dilated cardiomyopathy; *DSP*, desmoplakin; EDVi, end-diastolic volume indexed; EF, ejection fraction; ESVi, end-systolic volume indexed; *FLNC*, filamin C; GCS, global circumferential strain; GLS, global longitudinal strain; GRS, global radial strain; LA, left atrium; LV, left ventricle; MAPSE, mitral annular plane systolic excursion; MI, mass index; MWT, maximum wall thickness; RA, right atrium; RV, right ventricle; RWMA, regional wall motion abnormalities; TAPSE, tricuspid annular plane systolic excursion.

Discrete variables are presented as n (%) and continuous variables as mean ± SD or median (interquartile range) as appropriate.

* *P*-value for *DSP/FLNC* vs. other DCM genotypes. *P*-values < 0.05 are expressed in bold

# Supplementary Material

## Supplementary Methods

*Genetic analysis*

Genetic variants were identified by different sequencing technologies, using gene panels that reflect the standard practice at the time of testing, as previously published.[1] Current criteria for pathogenicity were reviewed for each selected variant, according to published reports in the ClinVar database,[2] Genome Aggregation Database (gnomAD)[3] minor allele frequency and recommendations by the American College of Medical Genetics and Genomics[4] (Supplementary Table 1).

*Clinical analysis*

Patients were considered symptomatic if there were any reports of dyspnea, chest pain and/or syncope. Family history was ascertained by the presence of at least one first or second-degree family member affected by DCM, AC, SCD or with previous documented episode(s) of otherwise unexplained sustained ventricular tachycardia (VT) or ventricular fibrillation.

*Cardiovascular magnetic resonance acquisition*

Pre-contrast breath-held steady-state free precession sequences were used to acquire cine images in standard long and short axis views. Late gadolinium enhancement (LGE) images (long and consecutive short axis slices) were acquired 10 minutes following a bolus administration of 0.1 mmol/kg gadolinium contrast agent (Gadoterate meglumine, Dotarem, Guerbet S.A., France) using a phase sensitive inversion recovery sequence. T1-weighted black blood imaging with and without fat suppression was performed in the 4-chamber view and mid LV short axis slice using double inversion recovery fast spin-echo sequence in 25 patients. Native pre-contrast T1 mapping was performed in 43 patients on

a mid-left ventricular short-axis slice using a modified Look-Locker inversion recovery sequence (all cases 1.5T Siemens Aera scanner).

*Statistical Analysis*

Statistical analyses were performed using SPSS statistical software (version 24.0, IBM Corp., Armonk, NY, USA). Discrete variables are presented as absolute frequencies with percentages; continuous as mean ± standard deviation if normally distributed, or median and interquartile ranges. Intra-observer (with a one-month temporal interval between repeat analyses) and inter-observer reproducibility were assessed on 10 random scans using intraclass correlation coefficients (Supplementary Table 2).

Differences between individual genotypes were assessed using one-way ANOVA for parametric data or Kruskal–Wallis test for nonparametric data; categorical variables were compared using Chi-square test. Significant effects (two-sided p-values <0.05) were further evaluated with post-hoc pairwise comparisons with Bonferroni adjustment.

All subjects were clustered post-hoc according to their genotype and imaging phenotype, the latter including LV end-diastolic volume indexed, LVEF, percentage of LGE in total LV mass (%LGE) and presence/absence of RV LGE. A two-step cluster analysis was performed with two predefined clusters and using a log-likelihood statistic as a distance measurement. All continuous variables in the model were standardized. The clustering feature tree had a maximum of 4 branches and 2 maximum levels of tree depth. The inputs weighting is described in detail in the Supplementary Figure 1. Grouped/clustered genotypes were then compared using Students' t-test or Mann-Whitney U test (parametric and nonparametric data respectively); categorical variables were compared using Fisher's exact test. Two-sided p-values <0.05 were considered significant.

**Supplementary Tables**

**Supplementary Table 1. Identified gene variants and classification.**

| Gene | Exonic and splice-site variants | gnomAD Minor Allele Frequency | ClinVar/ Reference | ACMG classification |
|---|---|---|---|---|
| *ACTC1* | C.569A>G (p.Tyr190Cys) | Not reported | Not reported | Likely to be pathogenic or disease-causing |
| *BAG3* | c.821C>A (p.Ser274*) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *BAG3* | c.925C>T (p.Arg309*) | Not reported | Pathogenic[5] | Pathogenic or disease-causing |
| *BAG3* | c.821C>A (p.Ser274*) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *DSP* | c.4477G>T (p.Glu1493*) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *DSP* | c.2572_2573insG (p.Glu858Glyfs*15) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *DSP* | c.5472delA (p.Asp1825Thrfs12*) | 3.982e-6 | Not reported | Likely to be pathogenic or disease-causing |
| *DSP* | k2091fs (p.Lys2091fs) | Not reported | Not reported | Probably associated with the disease |
| *DSP* | c.4531C>T (p.Gln1511*) | 2.001e-5 | Pathogenic[6] | Very likely to be pathogenic or disease-causing |

| | | | | |
|---|---|---|---|---|
| *DSP* | c.1267-2A>G | Not reported | Not reported | Likely to be pathogenic or disease-causing |
| *DSP* | c.7567_7570delAAGA (p.Lys2523GInfs*37) | Not reported | Pathogenic[7] | Pathogenic or disease-causing |
| *DSP* | c.8469_8487delGGGGTCCCGCTCCGGCTCC (p.Gly2824fs) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *DSP* | c.939+1G>A | 3.188e-5 | Pathogenic[8] | Very likely to be pathogenic or disease-causing |
| *DSP* | c.4025G>A (p.Trp1342*) | Not reported | Not reported | Pathogenic or disease-causing |
| *DSP* | c.3928A>T (p.Lys1310*) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *DSP* | c.1267-2A>G | Not reported | Not reported | Likely to be pathogenic or disease-causing |
| *DSP* | c.478C>T (p.Arg160*) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *DSP* | c.3G>T (p.Met1?) | Not reported | Not reported | Likely to be pathogenic or disease-causing |
| *DSP* | c.1288G>T (p.Glu430*) | Not reported | Not reported | Likely to be pathogenic or disease-causing |
| *DSP* | c.3928A>T (p.Lys1310*) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |

| | | | | |
|---|---|---|---|---|
| *DSP* | c.250C>T (p.Arg84*) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *DSP* | c.5875delT (p.Lys1626Argf*19) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *DSP* | c.3505T>A (p.Tyr1169Asn) | Not reported | Not reported | Likely to be pathogenic or disease-causing |
| *DSP* | c.2276_2277insA (p.Thr760Tyrfs*7) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *DSP* | c.5596C>T (p. Gln1866*) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *DSP* | c.8469_8487delGGGGTCCCGCTCCGGCTCC (p.Gly2824fs) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *DSP* | c.5596C>T (p. Gln1866*) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *DSP* | c.1288G>T (p.Glu430*) | Not reported | Not reported | Likely to be pathogenic or disease-causing |
| *DSP* | c.4875delT (p.Lys1626Argfs*19) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *FLNC* | c.1444C>T (p.Arg482*) | Not reported | Uncertain significance | Very likely to be pathogenic or disease-causing |
| *FLNC* | c.4718T>A (p.Leu1573*) | Not reported | Uncertain significance | Very likely to be pathogenic or disease-causing |

| | | | | |
|---|---|---|---|---|
| *FLNC* | c.2971C>T (p.Arg991*) | Not reported | Pathogenic[9] | Very likely to be pathogenic or disease-causing |
| *FLNC* | c.7252-1G >A | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *FLNC* | c.2971C>T (p.Arg991*) | Not reported | Pathogenic[9] | Very likely to be pathogenic or disease-causing |
| *FLNC* | c.2971C>T (p.Arg991*) | Not reported | Pathogenic[9] | Very likely to be pathogenic or disease-causing |
| *FLNC* | c.2971C>T (p.Arg991*) | Not reported | Pathogenic[9] | Very likely to be pathogenic or disease-causing |
| *LMNA* | c.1892dupG (p.Gly631fs) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *LMNA* | c.1489-1G>A | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *LMNA* | c.825_832delGCAGTCTG (p.Arg275SerfsX2) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *LMNA* | c.751dupC (p.Gln251fs) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *LMNA* | c.568C>T (p.Arg190Trp) | Not reported | Pathogenic[10] | Very likely to be pathogenic or disease-causing |
| *LMNA* | c.356+1G>C | Not reported | Pathogenic[11] | Very likely to be pathogenic or disease-causing |

| | | | | |
|---|---|---|---|---|
| *LMNA* | c.825_832delGCAGTCTG (p.Arg275SerfsX2) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *LMNA* | c.571delG (p.Val191Trpfs*10) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *LMNA* | c.1434dupG (p.Leu479Alafs) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *LMNA* | c.825_832delGCAGTCTG (p.Arg275SerfsX2) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *LMNA* | c.1434dupG (p.Leu479Alafs) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *LMNA* | c.73C>T (p.Arg25Cys) | Not reported | Not provided | Likely pathogenic or disease-causing |
| *MYBPC3* | c.748T>C (p.Ser250Pro) | Not reported | Not reported | Likely to be pathogenic or disease-causing |
| *MYH7* | c.602T>C (p.IIe201Thr) | 7.952e-6 | Likely pathogenic[12] | Likely pathogenic or disease-causing |
| *MYH7* | c.4498C>T (p.Arg1500Trp) | Not reported | Pathogenic/ likely pathogenic[13] | Very likely to be pathogenic or disease-causing |
| *MYH7* | c.379C>A (p.Pro127Thr) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *RBM20* | c.2176C>T (pArg726*) | Not reported | ClinVar-Uncertain significance | Very likely to be pathogenic or disease-causing |

| | | | | |
|---|---|---|---|---|
| *RBM20* | c.2639delA (p.Asn880Thrfs*30) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *RBM20* | c.1906C>T (p.Arg636Cys) | Not reported | Conflicting interpretations of pathogenicity | Likely to be pathogenic or disease-causing |
| *SCN5A* | c.665G>A (p.Arg222Gln) | Not reported | Pathogenic[14] | Pathogenic or disease-causing |
| *SCN5A* | c.3988G>A (p.Ala1330Thr) | Not reported | Pathogenic/ likely pathogenic[15] | Pathogenic or disease-causing |
| *TNNI3* | c65_66delGCinsTT (p.Arg22Leu) | Not reported | Not reported | Likely to be pathogenic or disease-causing |
| *TPM1* | c.359C>T (p.Ala120Val) | Not reported | Not reported | Likely to be pathogenic or disease-causing |
| *TTN* | c.32854C>T (p.Gln10952*) | Not reported | Uncertain significance | Very likely to be pathogenic or disease-causing |
| *TTN* | c.4239delT (p.Phe1413Leufs*3) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.64795delA (p.Arg21599Aspf*6) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.2645_26454delTTG insA (p.Trp8818Lysfs*6 ) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.76510A>T (p.Lys25504*) | Not reported | Not reported | Likely to be pathogenic or disease-causing |

| | | | | |
|---|---|---|---|---|
| *TTN* | c.58921C>T (p.Arg19641*) | Not reported | Pathogenic[16] | Likely to be pathogenic or disease-causing |
| *TTN* | c.55931G>A (p.Trp18644*) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.51784C>T (p.Arg1762*) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.25115_25116insTTGA (p.Glu8372Aspfs*2) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.25115_25116insTTGA (p.Glu8372Aspfs*2) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.20299C>T (p.Arg6767*) | Not reported | Pathogenic[17] | Very likely to be pathogenic or disease-causing |
| *TTN* | c.7389_73902delTAGT (p.Ser24634Metfs*9) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.65971C>T (p.Arg21991*) | Not reported | Likely Pathogenic[18] | Very likely to be pathogenic or disease-causing |
| *TTN* | c.36412delG (p.Ala12138Profs*44) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.18154+2T | Not reported | Not reported | Likely to be pathogenic or disease-causing |
| *TTN* | c.19730_19733delAACA (p.Lys6577Metfs*10) | Not reported | Pathogenic[19] | Likely to be pathogenic or disease-causing |

| | | | | |
|---|---|---|---|---|
| *TTN* | c.2099C>T (p.Arg6767*) | Not reported | Pathogenic[8] | Very likely to be pathogenic or disease-causing |
| *TTN* | c.33736C>T (p.Arg11246*) | Not reported | Likely Pathogenic[17, 18] | Very likely to be pathogenic or disease-causing |
| *TTN* | c.35830C>T (p.Arg11944*) | Not reported | Likely pathogenic[17,18,20] | Very likely to be pathogenic or disease-causing |
| *TTN* | c.18372_18373insTTAC (p.Val6125Leufs*18) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.35830C>T (p.Arg11944*) | Not reported | Likely pathogenic[17,18,20] | Very likely to be pathogenic or disease-causing |
| *TTN* | c.75592_75593delCC (p.Pro25198Serfs*3) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.24840_24841insTT (p.Leu8281Phefs*4) | Not reported | Likely pathogenic[21] | Very likely to be pathogenic or disease-causing |
| *TTN* | c.64795delA (p.Arg21599Aspf*6) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.64795delA (p.Arg21599Aspf*6) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.67813C>T (p.Arg22605*) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.18154+1G>T | Not reported | Not reported | Likely to be pathogenic or disease-causing |

| | | | | |
|---|---|---|---|---|
| *TTN* | c.25898_25899insG (p.Arg8634Serfs*17) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.64161_64162insT (p.Lys21388*) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |
| *TTN* | c.25115_25116insTTGA (p.Glu8372Aspfs*2) | Not reported | Not reported | Very likely to be pathogenic or disease-causing |

ACMG, American College of Medical Genetics and Genomics; *ACTC1*, alpha cardiac actin; *BAG3*, bcl2-associated athanogene 3; *DSP*, desmoplakin; *FLNC*, filamin; GnomAD, Genome Aggregation Database; *LMNA*, lamin A/C; *MYBPC3*, myosin-binding protein C; *MYH7*, beta-myosin heavy chain; *RBM20*, RNA binding motif protein 20; *SCN5A*, cardiac sodium channel $NA_v1.5$; *TPM1*, alpha-tropomyosin; *TNNI3*, cardiac troponin I; *TTN*, titin.

**Supplementary Table 2. Summary of reproducibility testing of CMR parameters**

| Parameter | Inter-observer ICC | Intra-observer ICC (E.N.) | Intra-observer ICC (S.M.F.) |
|---|---|---|---|
| LV EDV | 0.995 (0.978 – 0.999) | 0.995 (0.982 – 0.999) | 0.915 (0.695 – 0.978) |
| LV ESV | 0.987 (0.948 – 0.997) | 0.996 (0.986 – 0.999) | 0.974 (0.898 – 0.993) |
| LV mass | 0.989 (0.957 – 0.997) | 0.991 (0.965 – 0.998) | 0.940 (0.779 – 0.985) |
| RV EDV | 0.981 (0.925 – 0.995) | 0.981 (0.927 – 0.995) | 0.956 (0.834 – 0.989) |
| RV ESV | 0.975 (0.902 – 0.994) | 0.991 (0.965 – 0.998) | 0.918 (0.706 – 0.979) |
| LGE mass | 0.991 (0.964 – 0.998) | 0.994 (0.974 – 0.999) | 0.985 (0.942 – 0.996) |
| Native T1 mapping | 0.977 (0.911 – 0.994) | 0.970 (0.885 – 0.992) | 0.906 (0.643 – 0.978) |
| GRS | 0.929 (0.741 – 0.982) | 0.984 (0.922 – 0.997) | 0.737 (0.196 – 0.934) |
| GCS | 0.884 (0.602 – 0.970) | 0.873 (0.495 – 0.973) | 0.955 (0.813 – 0.990) |
| GLS | 0.884 (0.602 – 0.970) | 0.835 (0.381 – 0.965) | 0.876 (0.548 – 0.971) |

CMR, cardiovascular magnetic resonance; EDV, end-diastolic volume; ESVi, end-systolic volume; GCS, global circumferential strain; GLS, global longitudinal strain; GRS, global radial strain; ICC, intraclass correlation coefficient; LGE, late gadolinium enhancement; LV, left ventricle; RV, right ventricle.

Results presented with 95% confidence intervals.

Agreement was considered excellent when ICC > 0.74, good when ICC = 0.60–0.74, fair when ICC = 0.40–0.59 and poor when ICC < 0.40

Note: both observers reported a similar prevalence of RV LGE.

**Supplementary Table 3**. Demographic and clinical characteristics of the study population according to the individual gene.

| | ***TTN*** (n=30) | ***DSP*** (n=25) | ***LMNA*** (n=12) | ***FLNC*** (n=7) | ***BAG3*** (n=3) | ***RBM20*** (n=3) | ***SCN5A*** (n=2) | **Other Sarcomeric genes** (n=7) |
|---|---|---|---|---|---|---|---|---|
| **Demographic characteristics** | | | | | | | | |
| Male | 21 (70.0) | 11 (44.0) | 8 (66.7) | 4 (57.1) | 2 (66.7) | 0 | 1 (50.0) | 4 (57.1) |
| Age at diagnosis, years | 42.6 ± 12.4 | 40.5 ± 14.1 | 37.9 ± 15.4 | 48.9 ± 15.2 | 42.3 ± 11.6 | 45.0 ± 10.1 | 47.5 ± 4.9 | 44.0 ± 17.9 |
| Caucasian | 24 (80.0) | 23 (92.0) | 12 (100.0) | 7 (100.0) | 3 (100.0) | 3 (100.0) | 2 (100) | 7 (100) |
| Proband | 25 (83.3) | 20 (80.0) | 12 (100.0) | 5 (71.4) | 3 (100.0) | 3 (100.0) | 2 (100) | 6 (85.7) |
| **Clinical characteristics** | | | | | | | | |
| Symptoms | 16 (53.3) | 10 (40.0) | 4 (33.3) | 1 (14.3) | 0 | 2 (66.7) | 2 (100) | 3 (42.9) |
| Family history of DCM | 18 (60.0) | 11 (44.0) | 7 (58.3) | 1 (14.3) | 3 (100.0) | 2 (66.7) | 1 (50.0) | 0 |
| Family history of AC | 0* | 7 (28.0) | 0 | 4 (57.1) | 0 | 1 (33.3) | 0 | 0 |
| Family history of SCD / VT / VF | 8 (26.7) | 10 (40.0) | 7 (58.3) | 4 (57.1) | 1 (33.3) | 1 (33.3) | 1 (50.0) | 3 (42.9) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ICD | 6 (20.0) | 10 (40.0) | 8 (66.7) | 5 (71.4) | 0 | 2 (66.7) | 1 (50.0) | 0 |
| Primary prevention | 4 (66.7) | 8 (80.0) | 8 (100) | 3 (60.0) | 0 | 1 (50.0) | 0 | 0 |
| Medication | | | | | | | | |
| Beta-blocker | 27 (90.0) | 20 (80.0) | 6 (50.0) | 4 (57.1) | 2 (66.7) | 3 (100.0) | 2 (100) | 5 (71.4) |
| Anti-arrhythmic | 2 (6.7) | 0 | 2 (16.7) | 1 (14.3) | 1 (33.3) | 1 (33.3) | 1 (50.0) | 0 |
| ACEi / ARB | 26 (86.7) | 19 (76.0) | 6 (50.0) | 4 (57.1) | 3 (100.0) | 2 (66.7) | 2 (100) | 6 (85.7) |

ACEi, angiotensin-converting-enzyme inhibitor; ALVC, arrhythmogenic left ventricular cardiomyopathy; ARB, angiotensin II receptor blockers; *BAG3*, bcl2-associated athanogene 3; DCM, dilated cardiomyopathy; *DSP*, desmoplakin; *FLNC*, filamin C; ICD, implantable cardioverter-defibrillator; *LMNA*, lamin A/C; *RBM20*, RNA binding motif protein 20; SCD, sudden cardiac death; *SCN5A*, cardiac sodium channel $NA_v1.5$; *TTN,* titin; VF, ventricular fibrillation; VT, ventricular tachycardia.

Discrete variables are presented as n (%) and continuous variables as mean ± SD.

[*] *P-value* <0.002 vs. *FLNC*

**Supplementary Table 4**. Arrhythmic events and electrocardiographic characteristics of the study population according to individual genes.

| | ***TTN*** (n=30) | ***DSP*** (n=25) | ***LMNA*** (n=12) | ***FLNC*** (n=7) | ***BAG3*** (n=3) | ***RBM20*** (n=3) | ***SCN5A*** (n=2) | **Other Sarcomeric genes** (n=7) |
|---|---|---|---|---|---|---|---|---|
| TWI V2-V3 | 1 (3.3) | 4 (16.0) | 0 | 0 | 0 | 0 | 0 | 1 (14.3) |
| TWI V4-V6 | 3 (10.0) | 5 (20.0) | 0 | 1 (14.3) | 0 | 0 | 2 (100) | 1 (14.3) |
| LBBB | 1 (3.3) | 0 | 0 | 0 | 0 | 1 (33.3) | 0 | 0 |
| RBBB | 1 (3.3) | 1 (4.0) | 0 | 0 | 0 | 0 | 0 | 0 |
| ≥1,000 PVCs / 24h | 10 (33.3) | 12 (48.0) | 3 (25.0) | 3 (42.9) | 2 (66.7) | 1 (33.3) | 2 (100) | 4 (57.1) |
| ≥50 couplets / 24h | 2 (6.7) | 4 (16.0) | 2 (16.7) | 1 (14.3) | 0 | 1 (33.3) | 2 (100) | 1 (14.3) |
| Non-sustained VT | 13 (43.3) | 13 (52.0) | 4 (33.3) | 2 (28.6) | 2 (66.7) | 2 (66.7) | 2 (100) | 2 (28.6) |
| Sustained VT | 1 (3.3) | 0 | 0 | 1 (14.3) | 0 | 0 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| PVC / VT morphology | | | | | | | | |
| LBBB-like | 5 (16.7) | 1 (4.0) | 0 | 1 (14.3) | 0 | 0 | 0 | 0 |
| RBBB-like | 4 (13.3) | 0 | 2 (16.7) | 2 (28.6) | 1 (33.3) | 0 | 0 | 0 |
| Polymorphic | 4 (13.3) | 11 (44.0) | 1 (8.3) | 1 (14.3) | 1 (33.3) | 1 (33.3) | 2 (100) | 2 (28.6) |
| Aborted cardiac arrest | 1 (3.3) | 1 (4.0) | 0 | 1 (14.3) | 0 | 1 (33.3) | 1 (50.0) | 0 |
| ICD appropriate interventions | 0 | 0 | 1 (12.5) | 0 | 0 | 1 (50.0) | 0 | 0 |
| Major arrhythmias | 1 (3.3) | 3 (12.0) | 1 (8.3) | 3 (42.9) | 0 | 1 (33.3) | 1 (50.0) | 0 |
| Age at first arrhythmic event, years | 49.0 (16.0) | 36.0 (20.0) | 49.0 (17.0) | 52.0 (24.0) | 46.0 (3.0) | 51.0 (8.0) | 48.0 (6.0) | 62.0 (8.0) |

*BAG3*, bcl2-associated athanogene 3; *DSP*, desmoplakin; *FLNC*, filamin C; ICD, implantable cardioverter-defibrillator; LBBB, left bundle branch block; *LMNA*, lamin A/C; PVCs, premature ventricular contractions; RBBB, right bundle branch block; *RBM20*, RNA binding motif protein 20; *SCN5A*, cardiac sodium channel $NA_v1.5$; *TTN*, titin; TWI, T wave inversion; VF, ventricular fibrillation; VT, ventricular tachycardia.

Discrete variables are presented as n (%) and continuous variables as median (interquartile range).

Note: no significant differences were found between individual genotypes.

**Supplementary Table 5**. Imaging phenotypes in cardiac magnetic resonance according to individual genes.

| | ***TTN*** (n=30) | ***DSP*** (n=25) | ***LMNA*** (n=12) | ***FLNC*** (n=7) | ***BAG3*** (n=3) | ***RBM20*** (n=3) | ***SCN5A*** (n=2) | **Other Sarcomeric genes** (n=7) |
|---|---|---|---|---|---|---|---|---|
| LV EDVi, ml/m$^2$ | 97.0 (22.4) | 101.0 (34.0) | 94.3 (31.0) | 101.0 (17.0) | 96.0 (43.5) | 130.0 (17.5) | 110.2 (22.4) | 92.5 (92.9) |
| LV ESVi, ml/m$^2$ | 53.9 (19.0) | 52.9 (38.0) | 45.4 (23.9) | 41.0 (17.0) | 49.0 (13.3) | 73.7 (35.0) | 66.7 (25.3) | 48.6 (75.4) |
| LV CI, L/min | 3.2±0.7 | 3.1±0.7 | 3.1±0.6 | 3.1±0.8 | 3.0±0.7 | 2.4±0.3 | 3.6±0.6 | 2.7 ± 0.5 |
| LV EF, % | 43.1±10.4 | 48.6±13.4 | 48.7±12.7 | 50.7±7.3 | 52.0±3.0 | 36.9±11.1 | 40.3±8.0 | 40.1 ± 14.7 |
| LV RWMA | 2 (6.7) | 12 (48.0)$^*$ | 0$^\dagger$ | 3 (42.9) | 0 | 0 | 0 | 1 (14.3) |
| MAPSE, mm | 14.4±3.9 | 13.1±3.6 | 13.0±3.6 | 13.3±3.4 | 16.7±2.5 | 13.7±2.1 | 10.3 ± 5.3 | 10.4 ± 4.0 |
| LV MI, g/m$^2$ | 64.5±14.8 | 63.2±18.4 | 55.9±15.9 | 72.9±15.2 | 66.9±13.5 | 77.3±7.6 | 62.5±13.5 | 65.5±25.3 |
| MWT, mm | 9.7 (2.0) | 8.5 (2.0)$^*$ | 9.0 (2.5) | 9.0 (2.0) | 9.0 (3.2) | 11.0 (3.0) | 9.0 (6.0) | 7.0 (3.0)$^*$ |
| RV EDVi, ml/m$^2$ | 74.7 (35.1) | 87.2 (38.0) | 83.8 (12.5) | 95.0 (18.5) | 88.0 (37.0) | 77.1 (20.2) | 58.3 (32.6) | 97.1 (41.7) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| RV ESVi, $ml/m^2$ | 32.6 (27.0) | 40.5 (30.0) | 36.0 (5.9) | 44.0 (8.4) | 41.0 (15.0) | 42.9 (14.5) | 34.8 (2.0) | 50.1 (31.6) |
| RV CI, L/min | 3.0±0.9 | 3.1±0.8 | 3.0±0.6 | 2.9±0.6 | 3.0±0.7 | 2.4±0.6 | 3.4±0.7 | 2.9±0.4 |
| RV EF, % | 54.0 (10.0) | 53.0 (15.5) | 57.4 (14.0) | 54.0 (20.0) | 55.0 (3.0) | 52.5 (19.4) | 52.7 (1.3) | 49.0 (17.8) |
| RV RWMA | 0 | 9 (36.0)* | 0 | 1 (14.3) | 0 | 0 | 0 | 0 |
| TAPSE, mm | 19.1±5.0 | 22.4±4.6 | 18.9±4.4 | 21.6±4.3 | 24.4±7.5 | 18.4±2.3 | 17.5±2.1 | 16.9±4.7 |
| LA area, $cm^2$ | 25.7 (7.4) | 22.0 (7.2) | 24.5 (7.5) | 30.0 (5.8) | 31.0 (8.2) | 25.1 (8.4) | 23 (0) | 20.0 (12.8) |
| RA area, $cm^2$ | 23.9 (9.0) | 22.0 (7.3) | 25.0 (11.0) | 24.9 (8.0) | 23.3 (5.0) | 21.0 (4.5) | 16.5 (5.0) | 20.0 (8.0) |
| LV fat infiltration | 0 | 6 (37.5) | 0 | 0 | 0 | 0 | 0 | 0 |
| RV fat infiltration | 0 | 3 (18.8) | 0 | 0 | 0 | 0 | 0 | 0 |
| ARVC criteria | 0 | 5 (20.0) | 0 | 0 | 0 | 0 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ALVC features | 11 (36.7) | 13 (52.0) | 3 (25.0) | 4 (57.1) | 2 (66.7) | 1 (33.3) | 2 (100) | 3 (42.9) |
| Native T1 mapping, ms | 1056.9 (81.3) | 1025.9 (56.2) | 1003.7 (43.0) | 1041.0 (164.0) | 1010.1 (0.3) | 1016.1 (266.3) | 1082.0 (20.0) | 1014.5 (39.6) |
| LV GRS, % | 26.3 (16.3) | 28.9 (25.9) | 40.9 (18.9) | 31.5 (12.5) | 37.4 (9.6) | 31.3 (16.8) | 34.6 (32.2) | 28.7 (6.1) |
| LV GCS, % | -13.2 (7.4) | -14.8 (6.2) | -16.4 (6.4) | -17.1 (6.9) | -18.3 (3.0) | -9.7 (5.2) | -10.9 (1.5) | -14.7 (2.3) |
| LV GLS, % | -10.9 (2.9) | -12.9 (5.0) | -14.2 (8.3) | -19.3 (5.8) | -12.2 (6.6) | -8.4 (3.8) | -10.3 (0.3) | -11.5 (2.0) |

ALVC, arrhythmogenic left ventricular cardiomyopathy; ARVC, arrhythmogenic right ventricular cardiomyopathy; *BAG3*, bcl2-associated athanogene 3; CI, cardiac index; *DSP*, desmoplakin; EDVi, end-diastolic volume indexed; EF, ejection fraction; ESVi, end-systolic volume indexed; *FLNC*, filamin C; GCS, global circumferential strain; GLS, global longitudinal strain; GRS, global radial strain; LA, left atrium; *LMNA*, lamin A/C; LV, left ventricle; MAPSE, mitral annular plane systolic excursion; MI, mass index; MWT, maximum wall thickness; RA, right atrium; *RBM20*, RNA binding motif protein 20; RV, right ventricle; RWMA, regional wall motion abnormalities; *SCN5A*, cardiac sodium channel $NA_v1.5$; TAPSE, tricuspid annular plane systolic excursion; *TTN*, titin.

Discrete variables are presented as n (%) and continuous variables as mean ± SD or median (interquartile range) as appropriate.

* $P < 0.002$ vs. *TTN*

**Supplementary Table 6**. Late gadolinium enhancement according to aggregated and individual genotypes.

| | **Total population (n=89)** | ***DSP/FLNC*** | | | **Other DCM genotypes** | | | | | | | ***P* value** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Total (n=32)** | *DSP* (n=25) | *FLNC* (n=7) | **Total (n=57)** | *TTN* (n=30) | *LMNA* (n=12) | *BAG3* (n=3) | *RBM20* (n=3) | *SCN5A* (n=2) | Other sarcomeric genes (n=7) | |
| LV LGE | 68 (76.4) | 29 (90.6) | 23 (92.0) | 6 (85.7) | 39 (68.4) | 20 (66.7) | 9 (75.0) | 2 (66.7) | 3 (100) | 1 (50.0) | 4 (57.1) | **0.020** |
| LV LGE, g | 4.3 (13.9) | 16.5 (21.8) | 17.3 (24.1) | 15.9 (24.2) | 1.2 (4.6) | 1.3 (4.3)* | 1.6 (12.4)* | 0.7 (1.1)* | 4.3 (3.0) | 7.6 (15.2) | 0.4 (2.5)* | **<0.001** |
| LV LGE, % of LV mass | 3.0 (11.8) | 14.1 (11.1) | 16.2 (10.4) | 11.5 (18.7) | 1.1 (3.0) | 1.2 (2.9)* | 2.2 (8.6)* | 0.5 (0.7)* | 3.0 (3.0) | 6.2 (12.4) | 0.2 (2.1)* | **<0.001** |
| RV LGE | 8 (9.0) | 7 (21.9) | 6 (24.0) | 1 (14.3) | 1 (1.8) | 1 (3.3) | 0 | 0 | 0 | 0 | 0 | **0.003** |

*BAG3*, bcl2-associated athanogene 3; DCM, dilated cardiomyopathy; *DSP*, desmoplakin; *FLNC*, filamin C; LGE, late gadolinium enhancement; *LMNA*, lamin A/C; LV, left ventricle; *RBM20*, RNA binding motif protein 20; RV, right ventricle; *SCN5A*, cardiac sodium channel $NA_v1.5$; *TTN*, titin.

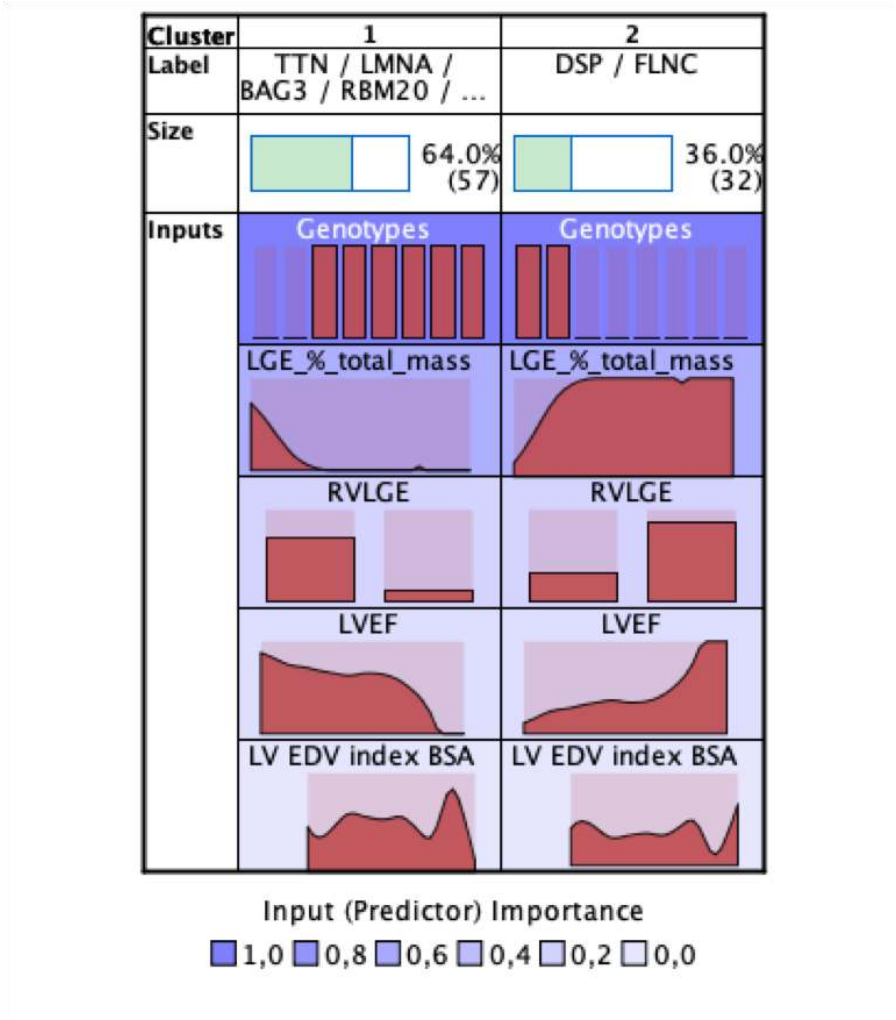
Discrete variables are presented as n (%) and continuous variables as median (interquartile range).

*P* value for *DSP/FLNC* vs. other DCM genotypes.

* $P < 0.002$ vs. *DSP*

**Supplementary Figures**

**Supplementary Figure 1**. Two-step cluster analysis. Five variables were used as inputs for clustering: the underlying genotypes, left ventricular (LV) end-diastolic volume indexed, LV ejection fraction, percentage of late gadolinium enhancement in total LV mass (%LGE) and presence/absence of right ventricular LGE. Two clusters were derived, one with both *DSP* and *FLNC* genotypes, the other with the remaining DCM genotypes. The clustering model is shown. Each variable/input is sorted in a descending order of importance (dark purple having the highest importance for the model, and light purple the least). Within each cell the relative distribution for each variable according to the cluster is presented.



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