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ORIGINAL RESEARCH ARTICLE

Desmoplakin Cardiomyopathy, a Fibrotic and Inflammatory Form of Cardiomyopathy Distinct From Typical Dilated or Arrhythmogenic Right Ventricular Cardiomyopathy

BACKGROUND: Mutations in desmoplakin (*DSP*), the primary force transducer between cardiac desmosomes and intermediate filaments, cause an arrhythmogenic form of cardiomyopathy that has been variably associated with arrhythmogenic right ventricular cardiomyopathy. Clinical correlates of *DSP* cardiomyopathy have been limited to small case series.

METHODS: Clinical and genetic data were collected on 107 patients with pathogenic *DSP* mutations and 81 patients with pathogenic plakophilin 2 (*PKP2*) mutations as a comparison cohort. A composite outcome of severe ventricular arrhythmia was assessed.

RESULTS: *DSP* and *PKP2* cohorts included similar proportions of probands (41% versus 42%) and patients with truncating mutations (98% versus 100%). Left ventricular (LV) predominant cardiomyopathy was exclusively present among patients with *DSP* (55% versus 0% for *PKP2*, $P<0.001$), whereas right ventricular cardiomyopathy was present in only 14% of patients with *DSP* versus 40% for *PKP2* ($P<0.001$). Arrhythmogenic right ventricular cardiomyopathy diagnostic criteria had poor sensitivity for *DSP* cardiomyopathy. LV late gadolinium enhancement was present in a primarily subepicardial distribution in 40% of patients with *DSP* (23/57 with magnetic resonance images). LV late gadolinium enhancement occurred with normal LV systolic function in 35% (8/23) of patients with *DSP*. Episodes of acute myocardial injury (chest pain with troponin elevation and normal coronary angiography) occurred in 15% of patients with *DSP* and were strongly associated with LV late gadolinium enhancement (90%), even in cases of acute myocardial injury with normal ventricular function (4/5, 80% with late gadolinium enhancement). In 4 *DSP* cases with 18F-fluorodeoxyglucose positron emission tomography scans, acute LV myocardial injury was associated with myocardial inflammation misdiagnosed initially as cardiac sarcoidosis or myocarditis. Left ventricle ejection fraction <55% was strongly associated with severe ventricular arrhythmias for *DSP* cases ($P<0.001$, sensitivity 85%, specificity 53%). Right ventricular ejection fraction <45% was associated with severe arrhythmias for *PKP2* cases ($P<0.001$) but was poorly associated for *DSP* cases ($P=0.8$). Frequent premature ventricular contractions were common among patients with severe arrhythmias for both *DSP* (80%) and *PKP2* (91%) groups (P=non-significant).

CONCLUSIONS: *DSP* cardiomyopathy is a distinct form of arrhythmogenic cardiomyopathy characterized by episodic myocardial injury, left ventricular fibrosis that precedes systolic dysfunction, and a high incidence of ventricular arrhythmias. A genotype-specific approach for diagnosis and risk stratification should be used.

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Clinical Perspective

What Is New?

- Desmoplakin (*DSP*) mutations cause a unique form of cardiomyopathy with a high prevalence of left ventricular (LV) fibrosis and systolic dysfunction.
- Although *DSP* cardiomyopathy shares a similar desmosomal molecular basis as plakophilin 2–associated arrhythmogenic right ventricular cardiomyopathy, diagnostic and risk stratification criteria that work well for plakophilin 2–associated arrhythmogenic right ventricular cardiomyopathy exhibit poor accuracy for *DSP* cardiomyopathy.
- Episodic myocardial injury in *DSP* cardiomyopathy contributes to progressive fibrosis that precedes the development of LV systolic dysfunction, an important difference compared with typical dilated cardiomyopathy.

What Are the Clinical Implications?

- Clinical diagnosis of *DSP* cardiomyopathy should rely on assessment of LV function, ventricular ectopy, and LV myocardial late gadolinium enhancement after genetic diagnosis.
- *DSP* cardiomyopathy should be considered in the differential diagnosis for acute myocardial inflammatory syndromes, including myocarditis and sarcoidosis.
- The presence of any LV systolic dysfunction in *DSP* cardiomyopathy (LV ejection fraction <55%), particularly when associated with frequent premature ventricular contractions and LV late gadolinium enhancement, indicates a substantial risk for severe ventricular arrhythmias.

Desmoplakin (DSP) is a structural protein that links the cardiac desmosome to intermediate filaments and is critical for normal force transmission in the myocardium.[1] Mutations in the *DSP* gene encoding desmoplakin were first identified in an autosomal recessive form of arrhythmogenic cardiomyopathy.[2] Case series established *DSP* mutations as an important cause of cardiomyopathy with a high predisposition toward ventricular arrhythmias.[3–5]

Because of its molecular role as a desmosomal binding protein and the high rate of arrhythmias, cardiomyopathy caused by *DSP* mutations has often been categorized as arrhythmogenic right ventricular (RV) cardiomyopathy (ARVC) or left dominant arrhythmogenic cardiomyopathy (LDAC). In contrast to typical ARVC, the concept of LDAC was introduced by Sen-Chowdhry et al in 2008[6] and most recently recognized by Corrado et al.[7] Multiple case series identified *DSP* mutations in LDAC.[3,5,8–12] In addition, 2 reports suggest a possible inflammatory component to the development and progression of *DSP* cardiomyopathy.[13,14] Moreover, a recent histology-based study of explanted arrhythmogenic cardiomyopathy hearts described a distinct pattern of pathological fibrosis in *DSP* cardiomyopathy hearts compared with typical ARVC hearts.[15] These previous studies suggest disease features of *DSP* cardiomyopathy different from typical forms of dilated cardiomyopathy (DCM) or ARVC. Although these small series have indicated different disease features for *DSP* cardiomyopathy, the prevalence of these findings has not been studied in a large cohort including both probands and family members. Moreover, the accuracy of diagnostic and risk stratification criteria developed for DCM and ARVC have not been investigated for *DSP* cardiomyopathy.

To systematically analyze the clinical spectrum of *DSP* cardiomyopathy, we developed a multicenter collaboration of cardiac genetics clinics to recruit patients with *DSP* mutations, including both probands and their genotype-positive family members. We similarly recruited patients with plakophilin 2 (*PKP2*) mutations as a comparison cohort representing typical ARVC. Our objectives were to determine the key clinical phenotypes and diagnostic features that distinguish *DSP* cardiomyopathy from typical DCM or ARVC. In the largest series of *DSP* mutation carriers reported to date, we find compelling evidence that *DSP* cardiomyopathy is a distinct form of cardiomyopathy, marked by a high proclivity for left ventricular fibrosis and arrhythmias, and associated with intermittent myocardial inflammatory episodes that appear clinically similar to myocarditis or sarcoidosis. Furthermore, we find that diagnostic and risk stratification variables that perform well for *PKP2*-associated ARVC exhibit poor accuracy for diagnosis and risk assessment for *DSP* mutation carriers. These results strongly indicate that a genotype-specific management approach is essential for *DSP* cardiomyopathy.

METHODS

The methods used are described for purposes of replicating the study procedure. Individual patient data will not be made available for purposes of reproducing the results. Patients with *DSP* and *PKP2* mutations were identified at 6 tertiary referral centers that routinely evaluate patients with both hereditary DCM and ARVC. Each center independently identified all patients for whom clinical genetic testing had been performed for DCM or ARVC. Both probands and family members with confirmed mutations were included to enable study of the variability in phenotypes of mutation carriers. Retrospective data were collected into a prespecified database. The data were combined into a master study database in a deidentified manner. The study was independently approved by the institutional review board at each center.

Genetic variant pathogenicity was assigned according to current criteria.[16] Pathogenic or likely pathogenic variants were considered as "mutations" and classified as

either truncating or missense mutations (truncating mutations defined as frameshift, nonsense, or splice site variants that result in premature termination codons). Of note, the "truncating" nomenclature is in keeping with the convention established for the DCM-associated gene, titin (*TTN*),[17] although actual truncated proteins may not be present in the case of mutations that cause premature termination codons, because the resultant mutant transcripts are typically degraded.[18]

Clinical data were collected from the initial clinic visit at each tertiary care center and included ECG, transthoracic echocardiography, magnetic resonance imaging (MRI), 18F-fluorodeoxyglucose positron emission tomography imaging, and rhythm monitoring when available. A subset of patients with MRI imaging were reviewed by a blinded cardiac MRI reader (P.A.) with expertise in both hereditary and nonhereditary cardiomyopathies. To compare clinical data similar to the typical categories of ARVC or LDAC, the cohort was divided into 3 groups as defined in the Methods in the Data Supplement: (1) normal ventricular function, (2) left ventricle (LV) predominant, and (3) RV predominant. The groups were further stratified based on the following variables: sex, age at evaluation, LV or RV dysfunction (left ventricle ejection fraction [LVEF] <50%, right ventricular ejection fraction ≤45% on transthoracic echocardiography/MRI or RV dysfunction visually by transthoracic echocardiography), thickening of the skin on the palms/soles (palmoplantar keratoderma) or curly hair, moderate (aerobic activity >30 min, >3× per week) or intense exercise (>60 min, >3× per week and beyond aerobic threshold) participation, LV enlargement (men: end diastolic volume by MRI >214 mL or left ventricular diastolic diameter >58 mm, women: end diastolic volume by MRI >178 mL or left ventricular diastolic diameter >52 mm),[19,20] presence of RV wall motion abnormality by MRI, evidence of T-wave inversions in at least 2 precordial leads on ECG, frequent premature ventricular contractions (PVCs), as defined by a PVC burden >500/24 h on Holter monitor, late gadolinium enhancement (LGE) in ≥2 myocardial segments on MRI, episodic chest pain as a primary symptom independent of arrhythmias, and significant troponin elevation (greater than upper limit of normal as per specific laboratory reference ranges) in the absence of obstructive coronary disease on coronary angiography. A composite severe arrhythmia outcome was defined as sudden cardiac death, sustained ventricular tachycardia, or appropriate implantable cardiac defibrillator therapy. Patients were assigned a possible, borderline, or definite diagnosis of ARVC as per the 2010 modified task force criteria.[21]

To analyze mutation distribution in comparison with common sequence variants, a literature review was performed to identify *DSP* cases. We individually reviewed each mutation, confirmed the sequence alteration from the *DSP* reference sequence, and confirmed classification of the mutations as truncating or missense. Evolutionary conservation at each locus (University of California Santa Cruz Genome Browser) was reported as number of sequence matches out of 100 mammalian reference sequences.[22] Because many variants were present only in single cases in the literature, pathogenicity cannot be assumed for individual cases. Common population variants for *DSP* were retrieved from the Genome Aggregation Database (gnomAD) and filtered for an allele frequency >4E-05 (K.J. Karczewski, PhD, unpublished data, 2019). In Table I in the Data Supplement, we separately categorized *DSP* variants reported in the literature as pathogenic but exceeding an allele frequency of 4E-05 as "Possibly Non-Pathogenic" variants, because this population frequency is greater than expected for deleterious autosomal dominant cardiomyopathy-causing mutations.[23] Therefore, this latter group was not included in the literature-reported missense mutations in Figure 1 and was not used for analysis of mutation clustering by domain.

Statistical Analysis

Continuous variables are reported as mean±SD and were compared with the Student *t* test or ANOVA for multiple groups with Tukey post hoc analysis. Categorical variables are reported as percentages and were compared with contingency tables and the Fisher exact test. Subgroup comparison tests were calculated using adjusted residuals and Bonferroni correction for multiple comparisons.[24] Event-free survival was estimated with the Kaplan-Meier method and compared with log-rank. Statistical analysis was performed using SPSS and Graphpad Prism.

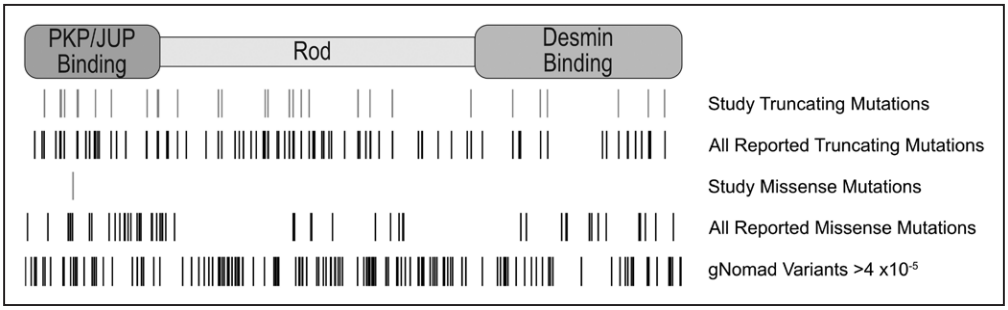

Figure 1. Mutation and population variant locations within desmoplakin (DSP).
Mutations present in the study cohort are shown in red for both truncating mutations (except large deletions, see Table II in the Data Supplement) and for missense mutations. All reported desmoplakin (*DSP*) mutations, including from this study, are shown in black. Missense mutations reported in the literature were only included if the allele frequency in the Genome Aggregation Database (gnomAD) population was $<4 \times 10^{-5}$ (all previously reported variants are included in Table I in the Data Supplement).[23] The bottom track shows all variants from the gnomAD population database with allele frequency $>4\times10^{-5}$—these variants are not expected to exert strong pathogenic effects based on tolerance in the general population.[23] *DSP* truncating mutations are dispersed throughout the gene. In contrast, missense mutations are enriched within the plakophilin/plakoglobin and desmin binding domains as compared with gnomAD population variants (70% vs 39% of variants; odds ratio, 3.6 [95% CI, 1.7–7.6], $P<0.001$).

RESULTS

Clinical Presentation of *DSP* Cardiomyopathy Is Distinct From *PKP2* Cardiomyopathy

The study included 107 patients with *DSP* mutations and 81 with *PKP2* mutations by commercially available genetic testing or obligate carrier status (*DSP* obligate carrier n=4). Characteristics of the overall cohort are summarized in Table 1. Mutations in both groups were predominantly truncating and predicted to result in allelic loss of function through nonsense mediated RNA decay (*DSP* n=105, *PKP2* n=81). Genetic variants are summarized in Tables II and III in the Data Supplement and Figure 1. The *DSP* cohort was 69% female with an average age at first evaluation of 36±16 years (versus 51% female for *PKP2*, *P*=0.01). LV predominant cardiomyopathy was exclusively present in *DSP* cardiomyopathy (52 of 103, 51%) as compared with *PKP2* cardiomyopathy (0 of 81, 0%). RV predominant cardiomyopathy was present in only 14 (14%) of patients with *DSP*, whereas it was uniformly present among patients with *PKP2* with overt cardiomyopathy (32 of 79, 40%, *P*<0.001). Fewer patients with *DSP* exhibited both normal LV and RV function as compared with patients with *PKP2* (36% versus 60%, *P*=0.002), indicating a significantly greater probability of overt cardiomyopathy (clinical penetrance) for *DSP* mutation carriers. Curly hair and/or thick skin on the palms or soles (palmoplantar keratoderma) was commonly present in patients with *DSP* (54/98, 55%) but not in patients with *PKP2* (1/46, 2%). T wave inversion in V1-3 was more common in patients with *PKP2* (50% versus 8%, *P*<0.001) and correlated with RV dysfunction (84% versus 27%, *P*<0.001; Table IV in the Data Supplement). Although T wave inversions were uncommon among patients with *DSP*, T wave inversions in V4-6 were more frequent with LV involvement (36% versus 3% with normal ventricular function, *P*=0.006). Patients with *DSP* with MRI data were significantly more likely to exhibit LV LGE (23 of 57, 40%) as compared with patients with *PKP2* (5 of 51, 10%, *P*<0.001). Episodic chest pain was more commonly reported by patients with *DSP* (21% versus 4% for *PKP2*, *P*=0.001) at an average age of 34±19 years. In 15 of these patients with *DSP*, acute myocardial injury (troponin elevations with no obstructive coronary disease by coronary angiography) was present, but no patients with *PKP2* had detected episodes of overt myocardial injury that presented with chest pain. The revised 2010 ARVC Task Force criteria were insensitive for clinical diagnosis in patients with *DSP* as compared with patients with *PKP2*—for example, only 34% of *DSP* cases met task force criteria for definite diagnosis versus 49% for *PKP2* cases (*P*=0.02), despite the greater overall cardiomyopathy penetrance with *DSP* (as above).

Table 1. Overall Cohort Characteristics

Overall Cohort Characteristics			
	***DSP*, n=107**	***PKP2*, n=81**	***P* Value**
Female	69%	51%	0.01
Age at evaluation, y	36±16	39±20	0.24
Proband	41%	42%	1.0
Normal ventricular function	36% (37/103)	60% (47/79)	0.002
RV predominant	14% (14/103)	40% (32/79)	<0.001
LV predominant	51% (52/103)	0% (0/79)	<0.001
Palmoplantar keratoderma or curly hair	55% (54/98)	2% (1/46)	<0.001
Moderate/intense exercise	53% (42/80)	NA	NA
Truncating mutation	98%	100%	0.51
TFC definite ARVC	34% (35/103)	49% (39/79)	0.036
TFC borderline ARVC	28% (29/103)	14% (11/79)	
TFC possible ARVC	38% (39/103)	37% (29/79)	
Episodic chest pain	21%	4%	0.001
Troponin elevation	15%	0%	<0.001
T wave inversions V1–V2	15% (15/101)	13% (10/76)	0.83
T wave inversions V1–V3	8% (8/101)	50% (38/76)	<0.001
T wave inversions V4–V6	21% (20/96)	22% (17/76)	0.85
Left bundle-branch block	1% (1/96)	0% (0/76)	1.0
Right bundle-branch block	2% (2/96)	1% (1/76)	1.0
Frequent PVCs (>500/24 h)	56% (32/57)	61% (23/38)	0.83
LV LGE	40% (23/57)	10% (5/51)	<0.001
LVEF, %	46±14, n=103	59±8, n=79	<0.001
VT outcome	28%	30%	0.87

Continuous values are reported as mean ± standard deviation.

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; *DSP*, desmoplakin; LGE, late gadolinium enhancement; LV, left ventricle; LVEF, left ventricle ejection fraction; *PKP2*, plakophilin 2; PVC, premature ventricular contraction; RV, right ventricle; TFC, task force criteria; and VT, ventricular tachycardia.

Left Dominant Arrhythmogenic Cardiomyopathy With Fibrosis Is the Primary Manifestation of *DSP* Cardiomyopathy

A total of 52 patients with *DSP* demonstrated primary LV involvement (79% of clinically affected patients with *DSP*, 51% of the total genotype-positive cohort), as shown in Table 2. Of the LV predominant *DSP* cases, RV systolic function was normal in 69%, and only 7% with MRIs (2/29) had evidence of RV wall motion abnormalities. Only 3 out of 52 (6%) of the patients in this group met major imaging criteria for an ARVC diagnosis. Precordial T wave inversions in V1-3 were insensitive for detecting cardiomyopathy in this group (9 of 50, 18%), whereas T wave inversions in V4-6 were more common (17 of 47, 36%). The overall sensitivity of ARVC task force diagnostic criteria was low in this group (only 42% with a definite ARVC diagnosis). Most patients with available Holter monitors had

Table 2. ***DSP*** **Cohort Clinical Characteristics Stratified by Ventricular Predominance Subgroup**

	Normal Ventricular Function (N=37)	Predominant RV Involvement (N=14)	Predominant LV Involvement (N=52)	*P* Value
Female	76%	57%	69%	0.48
Age at evaluation, y	32±14	46±16	35±16	0.02
Truncating mutation	95%	100%	100%	0.14
Palmoplantar keratoderma or curly hair	50% (18/36)	54%	61% (27/44)	0.63
Proband	10%	64%	54%	<0.001
Moderate/intense exercise	61% (14/23)	46% (5/11)	49% (22/45)	0.63
LV enlargement	6% (2/35) *P*<0.001	29% *P*=0.48	60% (30/50) *P*<0.001	<0.001
LV systolic dysfunction	0% *P*≤0.001	50% *P*=1.0	86% *P*≤0.001	<0.001
LVEF, %	68±5	50±10	37±13	<0.001
RV systolic dysfunction	0% *P*≤0.001	79% *P*≤0.001	31% *P*=0.09	<0.001
RV focal WMA	0% (0/19) *P*=0.021	64% (7/11) *P*<0.001	8% (2/26) *P*=0.11	<0.001
ECG T wave inversions (V1–V2)	15% (5/34)	7% (1/14)	18% (9/50)	0.60
ECG T wave inversions (V1–V3)	3% (1/34)	7% (1/14)	12% (6/50)	0.34
ECG T wave inversions (V4–V6)	3% (1/32) *P*≤0.001	14% (2/14) *P*=0.09	36% (17/47) *P*=0.005	0.006
Frequent PVCs (>500/24 h)	29% (6/21) *P*=0.001	71% (5/7) *P*=0.37	72% (21/29) *P*=0.012	0.006
Episodic chest pain	14%	14%	29%	0.18
Acute myocardial injury*	8%	14%	21%	0.24
LV LGE	0% (0/19) *P*<0.001	27% (3/11) *P*=0.31	74% (20/27) *P*<0.001	<0.001
RV EDV, mL (MRI)	128±31 (n=18)	181±44 (n=10)	153±48 (n=22)	0.009
LV EDV, mL (MRI)	134±33 (n=18)	170±40 (n=10)	172±54 (n=24)	0.02
ARVC task force diagnosis				0.001
Possible	68% *P*≤0.001	22% *P*=0.17	21% *P*≤0.001	
Borderline	19% *P*=0.12	21% *P*=0.55	37% *P*=0.056	
Definite	13% *P*=0.001	57% *P*=0.049	42% *P*=0.071	
VT outcome	13.5% *P*=0.001	21% *P*=0.009	37% *P*=0.11	0.048

Continuous values are reported as mean ± standard deviation.

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; *DSP*, desmoplakin; EDV, end diastolic volume; LGE, late gadolinium enhancement; LV, left ventricle; LVEF, left ventricle ejection fraction; MRI, magnetic resonance imaging; PVC, premature ventricular contraction; RV, right ventricle; VT, ventricular tachycardia; and WMA, wall motion abnormality.

*Acute myocardial injury was defined as acute chest pain episode with documented troponin elevation and normal coronary angiography at the time of chest pain presentation.

frequent PVCs (21 of 29, 72%), consistent with an arrhythmogenic substrate.

Of patients with LV predominant cardiomyopathy, 74% of patients with available MRIs (20/27) exhibited LV LGE (Table 2). LV LGE occurred in 26% (7/27) in the absence of overt LV systolic dysfunction. In a blinded review of 10 cases, the pattern of fibrosis was characteristically subepicardial in the inferior segment in all 10 cases with extension to the midmyocardium in the septum in some cases (Figure 2, Figure I in the Data Supplement). Circumferential LGE with a primarily subepicardial distribution was present in 2 of 10 cases. The presence of intramyocardial fat adjacent to fibrosis was present in 4 of the 10 cases. The distribution of intramyocardial fat in or adjacent to regions of subepicardial fibrosis is highly similar to a recent report of histopathologic analyses on autopsy samples from sudden death cases associated with LDAC.[15] None of the 10 cases from our review demonstrated evidence of RV fat or fibrosis, also consistent with the recent autopsy series from LDAC cases.[25]

A Subset of Patients With *DSP* Exhibits Predominant RV Cardiomyopathy, Most Often With Concomitant LV Involvement

A comparison of the LV predominant patients with *DSP* with the minority of RV predominant patients (14 of 103, 14%) is shown in Table 2. These patients were diagnosed later in life when compared with the LV predominant group (mean age of diagnosis: 47±15 years versus 35±15 years, *P*=0.03). Although the ARVC task force criteria for a definite diagnosis were more often positive in this group, they were still insensitive given the presence of clinically confirmed cardiomyopathy (57% versus 42% for LV predominant, *P*=0.001). ECG

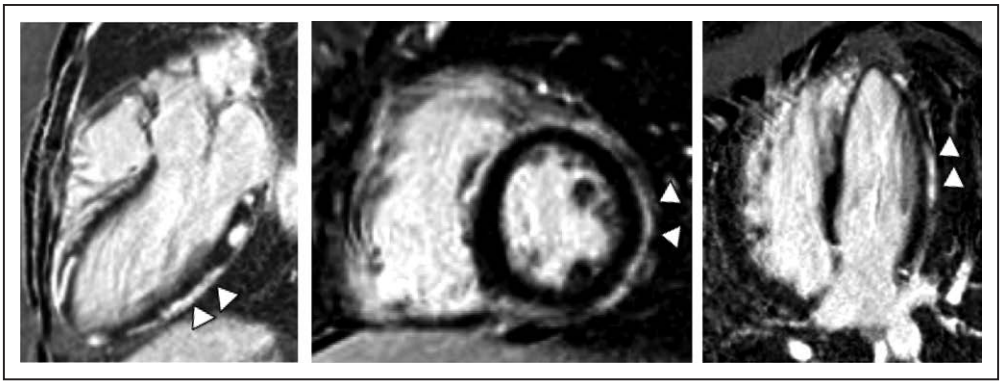

Figure 2. Acute myocardial injury and fibrosis preceding systolic dysfunction.
Cardiac magnetic resonance imaging (MRI) was performed in a 19-year-old patient who presented with recurrent episodes of chest pain, troponin elevation, and normal coronary angiography. The MRI revealed extensive left ventricular (LV) late gadolinium enhancement but normal LV systolic function (ejection fraction 60%). Right ventricular function was also normal without focal wall motion abnormalities. A family history of sudden cardiac death (mother and maternal uncle) prompted genetic testing, which revealed a pathogenic frameshift mutation in *DSP* (c.2593_2594dupGA).

criteria for ARVC also demonstrated modest sensitivity for the RV predominant group (minor criterion positive in 6 of 14, 43%; major criterion positive in 2 of 14, 14%). In comparison, patients with *PKP2* with overt RV cardiomyopathy exhibited precordial T wave inversions in V1-3 in 84% of cases (26 of 31, *P*<0.001; Table IV in the Data Supplement).

Concomitant LV dysfunction was present in 7 of the 14 RV predominant patients with *DSP* (50%), and 3 of these cases had LV LGE when MRIs were performed. The primary MRI imaging was available for review in 1 of these cases, and LV LGE was primarily subepicardial in the inferior and inferoseptal segments—a similar pattern of LV LGE as with patients with left dominant disease. Only 4 out of the entire *DSP* cohort (4%) had apparent isolated RV cardiomyopathy, a marked contrast from the *PKP2* cohort. In the *PKP2* cohort, LV involvement was only present in 5 of 81 cases (6%), and all of these cases occurred in the context of RV predominant cardiomyopathy (Table IV in the Data Supplement). Thus, apparently isolated RV cardiomyopathy was by far the most common presentation for *PKP2* cardiomyopathy (84% of clinically affected *PKP2* patients, *P*<0.001 versus *DSP*).

Myocardial Inflammatory Episodes

Acute myocardial injury was observed in 15% of patients with *DSP* (16/107) without a significant difference among the 3 clinically defined groups (Table 2). MRI data were available for 10 of these patients with 90% demonstrating LGE in the LV. Four of the patients with troponin elevation also underwent cardiac ^{18}F-fluorodeoxyglucose positron emission tomography, which showed evidence of active inflammation in the myocardium (Figure 3, Figures II and III in the Data Supplement). Of these 16 patients, 7/16 (44%) exhibited LV systolic dysfunction, and 1/16 (6%) exhibited both RV and LV systolic dysfunction. Of the 7 patients with normal ventricular systolic function and MRIs, 6 had evidence of LV LGE. In 1 of these cases with acute myocardial injury and normal ventricular systolic function, LV LGE was extensive (Figure 2).

Effect of Sex and Exercise in *DSP* Cardiomyopathy

A larger proportion of the *DSP* cohort was female as compared with the *PKP2* cohort (69% versus 51%, *P*=0.01). Therefore, we performed additional analyses to determine whether the greater proportion of females might be a result of an effect of sex on disease burden. As shown in Table V in the Data Supplement, there was no significant difference between any of the clinical variables compared.

Because vigorous exercise has been implicated in the pathogenesis of ARVC,[26] we compared exercise and sports history among 80 patients with *DSP* for whom these data were available. Moderate to intense exercise was reported in 53% (42/80) of patients. There was no difference in exercise participation between normal ventricular function and left or right dominant cardiomyopathy groups (*P*=0.515). In contrast to expectation, a smaller proportion of probands reported a moderate or intense exercise history (35%, 11/31 versus 63%, 31/49, *P*=0.02). Exercise history was not associated with a greater risk for experiencing severe ventricular arrhythmias among patients with *DSP* (*P*=0.14).

Clinical Spectrum of *DSP* Cardiomyopathy Among Probands and Nonprobands

To establish the phenotypic spectrum and estimate the penetrance of *DSP* mutations, we compared *DSP* cardiomyopathy probands to their genotype-positive family members (Table 3). The nonproband *DSP* group demonstrated a smaller proportion of patients with LV dysfunction (33% versus 74%, *P*<0.001) and higher LV ejection fraction (53% versus 38%, *P*<0.001) and were less likely to have T-wave inversions in leads V4-6 (7% versus 39%, *P*<0.001). Despite the nonproband group demonstrating

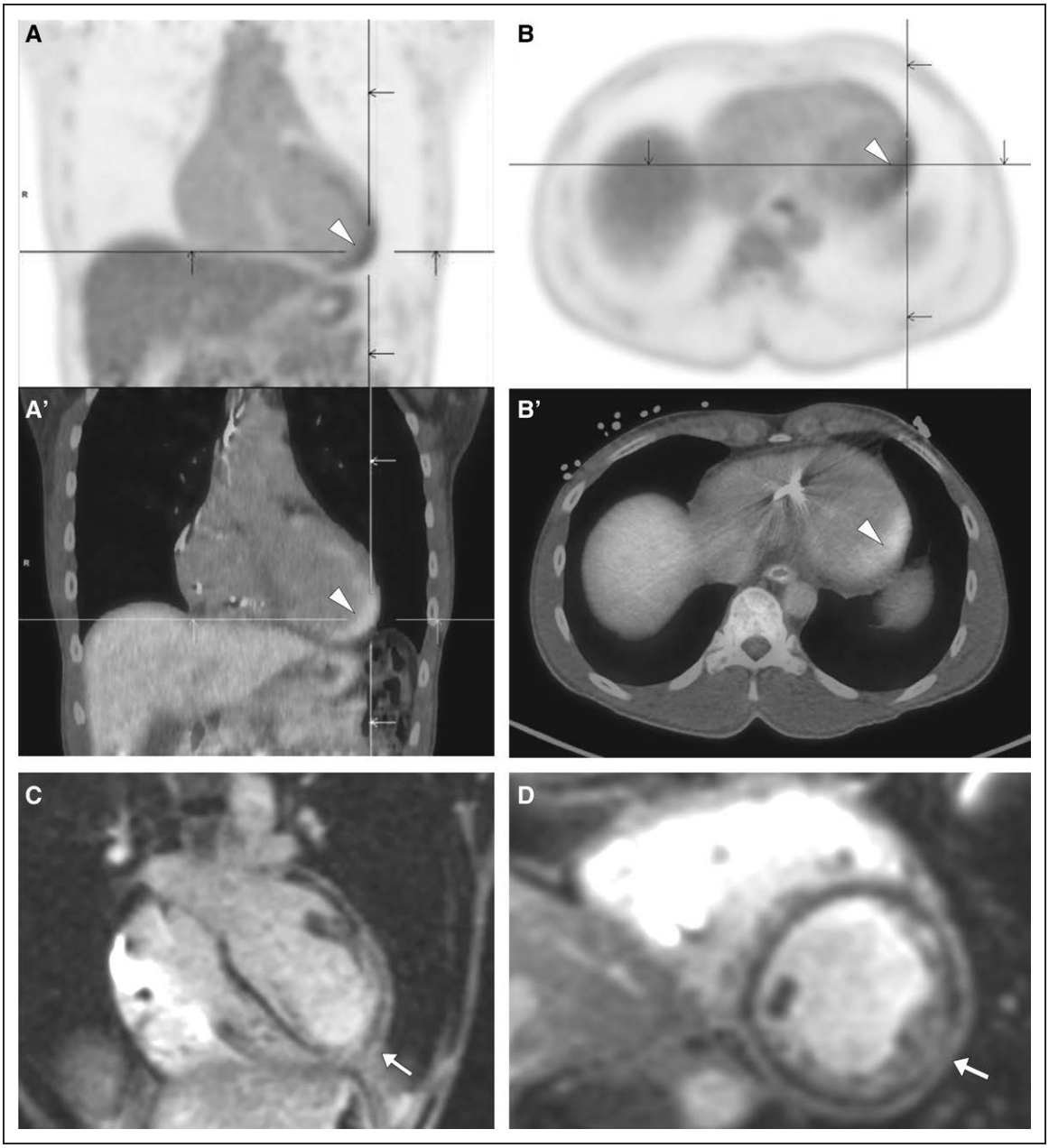

Figure 3. Acute myocardial inflammation in a desmoplakin (*DSP*) **cardiomyopathy patient presenting with acute myocardial injury**.
Images were obtained from a 41-year-old man with a desmoplakin truncating mutation (p.Arg2284*) after presentation with acute chest pain, troponin elevation, and normal coronary angiography. **A**, ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) and FDG-PET/computed tomography (CT; **A'**) in the coronal view. **B**, FDG-PET and FDG-PET/CT (**B'**) in the axial view. Arrowheads indicate areas of inflammation detected by FDG uptake. **C** and **D**, Cardiac magnetic resonance in the 4-chamber view (**C**) and short-axis view (**D**) demonstrated late gadolinium enhancement in a subepicardial distribution, including regions corresponding to increased inflammation (arrows). This patient also exhibited reduced left ventricular systolic function with an ejection fraction of 45%.

more individuals with normal LV function than the proband group, a high proportion of nonprobands exhibited frequent PVCs (43% with PVC number >500/24 h). The proportion of *DSP* nonprobands who presented with chest pain, troponin elevation, and LV LGE was similar to probands. The overall penetrance of evident cardiomyopathy, frequent PVCs, sustained ventricular tachycardia, and myocardial inflammatory episodes in the nonproband *DSP* group was 57% by a mean age of 40±16 years. The penetrance of overt cardiomyopathy was significantly lower in nonproband patients with *PKP2* (28% versus 57%, *P*=0.003). Although the *DSP* proband group was more likely to experience the combined ventricular tachycardia outcome, the composite severe ventricular arrhythmia outcome was still relatively common within the nonproband *DSP* group (43% versus 18%, *P*=0.005). This analysis highlights that (1) the family members of patients with *DSP* carry a substantial risk of adverse events, and (2) the most sensitive tests for clinical diagnosis in *DSP* mutation carriers are cardiac MRI (with assessment of LV LGE, regardless of LV function by echocardiogram),

Table 3. Proband Versus Nonproband Characteristics

	DSP			*PKP2*		
	Nonproband, N=63	Proband, N=44	*P* Value	Nonproband, N=47	Proband, N=34	*P* Value
Female	70%	68%	1.00	51%	49%	0.26
Age at evaluation, y	36±17	35±15	0.73	39±22	39±18	0.95
Palmoplantar keratoderma or curly hair	61% (35/57)	46% (19/41)	0.15	0% (0/23)	4% (1/23)	1.00
Moderate/intense exercise	63% (31/49)	35% (11/31)	0.02	NA	NA	NA
LV enlargement	29% (17/59)	46% (19/41)	0.09	13% (5/45)	9% (3/34)	1.00
LV systolic dysfunction	33% (20/61)	74% (31/42)	<0.001	7% (3/45)	6% (2/34)	1.00
LVEF, %	53±11	38±14	<0.001	59±10 (n=45)	59±5	0.76
RV systolic dysfunction	8% (5/61)	26% (11/42)	0.024	16% (7/45)	59% (20/34)	<0.001
RV focal WMA	8% (3/37)	28% (5/18)	0.10	23% (7/30)	43% (9/21)	0.220
ECG T wave inversions (V1–V2)	12% (7/59)	19% (8/42)	0.40	12% (5/42)	15% (5/34)	0.74
ECG T wave inversions (V1–V3)	5% (3/59)	12% (5/42)	0.27	29% (12/42)	77% (26/34)	<0.001
ECG T wave inversions (V4–V6)	7% (4/55)	39% (16/41)	<0.001	14% (6/42)	32% (11/34)	0.09
Frequent PVCs (>500/24 h)	43% (16/37)	80% (16/20)	0.01	39% (9/23)	14/15 (93%)	0.002
Episodic chest pain	18%	25%	0.46	2%	6%	0.57
Troponin elevation	13%	18%	0.58	0%	0%	1.0
LV LGE	43% (15/35)	36% (8/22)	0.78	10% (3/29)	9% (2/22)	1.0
RV EDV, mL (MRI)	145±45 (n=34)	160±46 (n=16)	0.28	169±63 (n=28)	220±48 (n=18)	0.006
LV EDV, mL (MRI)	155±53 (n=35)	166±37 (n=17)	0.46	157±68 (n=29)	152±30 (n=21)	0.78
VT Outcome	18%	43%	0.005	6%	62%	<0.001

Continuous values are reported as mean ± standard deviation.

DSP indicates desmoplakin; EDV, end diastolic volume; LGE, late gadolinium enhancement; LV, left ventricle; LVEF, left ventricle ejection fraction; MRI, magnetic resonance imaging; NA, not available; *PKP2*, plakophilin 2; PVC, premature ventricular contraction; RV, right ventricle; VT, ventricular tachycardia; and WMA, wall motion abnormality.

rhythm monitoring for PVC burden assessment, and troponin level (if acute chest pain).

Truncating *DSP* Mutations Are Homogeneously Distributed, Whereas Missense Mutations Cluster in Protein-Binding Domains

Specific mutation types and loci within the *DSP* gene coding sequence are shown in Figure 1 and listed in Table II in the Data Supplement. We also collated other reported *DSP* mutations from the literature to more comprehensively analyze whether mutations cluster in specific regions (Figure 1, Table I in the Data Supplement). Mutations in this study were primarily truncating mutations (ie, frameshift, nonsense, or splice site mutations). Truncating mutations in this and other studies occur throughout the gene without evident clustering. In contrast, missense mutations were more likely to involve the plakophilin/plakoglobin and desmin binding domains as compared with relatively common gnomAD variants (70% versus 39%; odds ratio, 3.6 [95% CI, 1.7–7.6], $P<0.001$).[23,27]

Severe Ventricular Arrhythmia Outcome Analysis

The combined outcome of sustained ventricular tachycardia, sudden cardiac death, or appropriate implantable cardiac defibrillator therapy occurred in 28% (30/107) of patients with *DSP* (N=19, 4, 7, respectively, for the individual outcomes) and 30% (24/81) of patients with *PKP2* (*P*=non-significant). In univariate analysis, LVEF <55% was strongly associated with events for *DSP*, whereas RV dysfunction was strongly associated with events for *PKP2* (Table 4). An LVEF threshold of <35% failed to identify 14 of 27 (52%) of patients with severe arrhythmias, whereas an LVEF threshold of <55% failed to identify 4 of 27 (15%). Based on the genotype-specific univariate analysis results, Kaplan-Meier time to event analysis was performed stratified by LV systolic dysfunction for the *DSP* cohort and by RV systolic dysfunction for the *PKP2* cohort, confirming the prognostic importance of these variables in the independent cohorts (Figure 4). A subset of patients with *DSP* with normal biventricular function also demonstrated the primary outcome with some events occurring before 30 years of age. Only 2 out of 4 (50%) individuals who had events and were classified as normal ventricular function had cardiac MRIs performed, so the

Table 4. Univariate Analysis of Variables for Association With the Combined Severe Ventricular Arrhythmia Outcome

	No Severe Arrhythmia	Severe Arrhythmia	*P* Value	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
DSP							
LVEF <55%	47% (36/76)	85% (23/27)	0.001	85%	53%	39%	91%
LVEF <35%	17% (13/76)	48% (13/27)	0.004	48%	83%	50%	85%
RV dysfunction	30% (23/76)	33% (9/27)	0.81				
LV LGE	34% (15/44)	50% (6/12)	0.35				
Frequent PVCs	51% (24/47)	80% (8/10)	0.16				
Moderate/intense exercise	58% (34/59)	38% (8/21)	0.137				
Acute myocardial injury episodes	21%	20%	0.93				
PKP2							
LVEF <55%	9% (5/55)	21% (5/24)	0.27				
LVEF <35%	4% (2/55)	0% (0/24)	0.57				
RV dysfunction	18% (10/55)	71% (17/24)	<0.001	71%	82%	63%	86%
LV LGE	6% (2/36)	20% (3/15)	0.14				
Frequent PVCs	48% (13/27)	91% (10/11)	0.03	91%	52%	43%	93%

DSP indicates desmoplakin; LGE, late gadolinium enhancement; LV, left ventricle; LVEF, left ventricle ejection fraction; *PKP2*, plakophilin 2; PVC, premature ventricular contraction; and RV, right ventricle.

presence of fibrosis is unknown in these patients. Frequent PVCs were highly prevalent among both patients with *DSP* (80%) and *PKP2* (91%) who experienced severe ventricular arrhythmias (*P*=NS, Table 4).

DISCUSSION

Cardiomyopathies have been classified into major clinical categories to enable development of generalized diagnostic, management, and risk stratification strategies. This approach has led to improved identification of robust risk factors specific to major categories, such as DCM and ARVC. The emergence of clinical genetic testing now enables a further step toward molecular subclassification of cardiomyopathy types, and an opportunity to reappraise the clinical accuracy of these classification strategies. *DSP* mutations have been reported to cause DCM and LDAC,[6,8,28,29] but also included in ARVC cohorts,[30–33] leading to a lack of clarity in whether and how to apply diagnostic, risk, and management strategies to *DSP* mutation carriers. In this context, we performed a gene-centered analysis of the clinical spectrum, natural history, and risk predictors for the largest cohort of *DSP* mutation carriers to date and found that a molecular diagnosis is essential to the correct identification and management of these patients.

Defining Features and Clinical Diagnosis of *DSP* Cardiomyopathy

Small case series have described an LV predominant cardiomyopathy caused by *DSP* mutations, but the

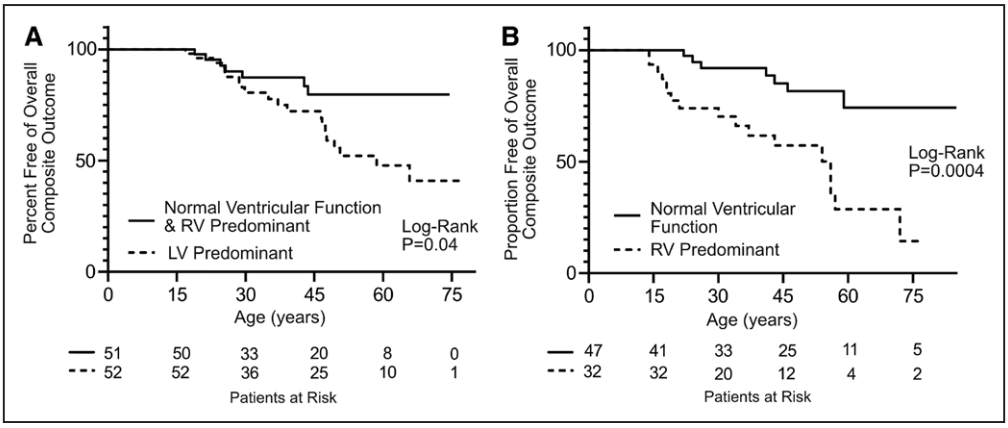

Figure 4. Survival analysis of severe ventricular arrhythmia outcomes.
A, Desmoplakin mutation carrier survival stratified by normal ventricular function and RV systolic dysfunction vs LV systolic dysfunction groups. **B**, Plakophilin-2 mutation carrier survival stratified by normal ventricular function and RV systolic dysfunction groups. LV indicates left ventricle; and RV, right ventricle.

prevalence and variability of this finding have not been previously examined in a large cohort.[6] Here, we found that *DSP* cardiomyopathy involves the LV in almost all cases and often without any apparent RV involvement. This finding was in clear contrast to *PKP2* cardiomyopathy, which always involved the RV predominantly and most often in isolation. Not surprisingly, the ARVC task force criteria, validated primarily in *PKP2*-enriched cohorts, performed poorly for detection of clinically affected *DSP* probands and nonprobands. Even among patients with *DSP* cardiomyopathy with apparent RV predominant cardiomyopathy, T wave inversions exhibited poor sensitivity. In addition to LV systolic dysfunction, we identified cases in which significant LV fibrosis and frequent PVCs preexisted overt LV systolic dysfunction or LV enlargement, a key distinction from typical DCM.[34,35] Taken together, these findings suggest that the primary, and most sensitive, clinical signs of *DSP* cardiomyopathy are LV systolic dysfunction, LV fibrosis on MRI, and frequent PVCs. The presence of curly hair and thick callused skin on the hands and soles of the feet (palmoplantar keratoderma) further heightens the likelihood of *DSP* cardiomyopathy, as recently reported for autosomal dominant *DSP* mutations.[18] These findings were also common in our cohort (Table 1), though the sensitivity and extent of these findings were not fully characterized in this report.

Recurrent LV Inflammation and Fibrosis as Primary Disease Mechanisms in *DSP* Cardiomyopathy

The presence of acute myocardial injury episodes among *DSP* mutation carriers has been suggested by previous case reports and case series,[13,14,36,37] but the frequency of this finding and association with disease stage have not been clear in the absence of data from large cohorts that include nonprobands. Here, we find that episodes of clinically apparent myocardial injury are common among probands (18%) and nonprobands (13%). Moreover, we find that acute episodes of myocardial injury occur even in the presence of normal systolic function, suggesting that myocardial injury and fibrosis precede overt systolic dysfunction in *DSP* cardiomyopathy (Figure 2). We used strict criteria to define acute myocardial injury (requiring troponin elevation with normal coronary angiography), likely leading to underestimation of the true prevalence of this phenomenon in the progression of the disease. In 4 of these cases, we found that episodes of injury were associated with cardiac ^{18}F-fluorodeoxyglucose positron emission tomography scans that showed typical findings for myocardial inflammation in the LV. Consistent with this observation, evidence of inflammatory infiltrates and scarring has been demonstrated previously in limited histological analyses of *DSP* patient LV myocardium.[5] Interestingly, in contrast to the *DSP* group in our study, none of the patients with *PKP2* in this study had documented episodes of acute myocardial injury, despite reports of myocardial inflammation as a component of typical ARVC.[32,38] We speculate that episodic RV myocardial inflammation in ARVC may be less likely to cause episodic chest pain that leads to clinical recognition, as compared with predominant LV inflammation in patients with *DSP*. Modulation of inflammatory signaling pathways may prove to be a novel therapeutic target for desmosomal-mediated cardiomyopathy, as recently demonstrated in a mouse model harboring homozygous mutations in *DSG2*.[39] Our findings here indicate that the window of opportunity for potential inflammatory modulation may be at an early disease stage, preceding development of LV systolic dysfunction.

In addition, 90% of cases of acute myocardial injury with available MRIs and 53% of all clinically affected *DSP* cases exhibited late gadolinium enhancement consistent with LV fibrosis. A specific pattern of LGE was present, occurring mainly in the LV subepicardium rather than mid-myocardial as is often seen with nonischemic cardiomyopathy.[40,41] These imaging findings are consistent with a recent histopathologic analysis that showed a distinct fibrosis pattern with *DSP* mutations compared with *PKP2* mutations.[15] Taken together, these observations implicate acute myocardial inflammatory episodes as a proximal cause of myocardial fibrosis and progressive dysfunction in the presence of *DSP* mutations. Our findings also strongly suggest that *DSP* cardiomyopathy should be considered in the differential diagnosis of other inflammatory myocardial processes, namely cardiac sarcoidosis and myocarditis.

DSP Cardiomyopathy Progression Is Independent of Sex or Exercise History

We also examined whether penetrance and variable expressivity could be related to sex and exercise history. In ARVC cohorts with high proportions of *PKP2* mutation carriers, both male sex[42] and a history of strenuous exercise[26] have been linked to disease progression and ventricular arrhythmias. Interestingly, our *DSP* cohort had a substantially greater proportion of females (69%) than the *PKP2* cohort (51%). Yet, comparison between males and females with *DSP* mutations did not reveal evidence of a greater disease burden among females. Although this finding requires replication in other substantially sized *DSP* cohorts, it indicates that male sex should not be considered a risk factor for ventricular arrhythmia risk stratification for *DSP* cardiomyopathy, as it has been for ARVC.[31,43,44] In the present cohort, 53% (42/80) of patients participated in at least moderate exercise without a significant difference in ventricular dysfunction or arrhythmias between exercise groups.

Paradoxically, a moderate or vigorous exercise history was less commonly reported by probands, who exhibited a greater disease burden. Although this finding is in contrast to reports for ARVC, it is consistent with a recent study of treadmill exercise in heterozygous *DSP* truncation mice, which showed evidence of improved inflammatory and other dysregulated cell-signaling markers after sustained exercise training (although a similar level of myocardial dysfunction persisted regardless of exercise group).[45] Taken together, our findings do not suggest an obvious interaction between exercise intensity and *DSP* cardiomyopathy severity, and, therefore, indicate further clinical studies should be performed before creating strict guidelines on recommended exercise levels for patients with *DSP*.

Risk Stratification for Severe Ventricular Arrhythmias Requires a Genotype-Specific Approach for *DSP* Cardiomyopathy

DSP cardiomyopathy has been associated with a high prevalence of ventricular arrhythmias since early descriptions.[3,5,11] However, risk factors specific to *DSP* cardiomyopathy have not previously been reported. We demonstrate here that risk stratification on the basis of variables studied in DCM or ARVC populations are inadequate for risk estimation in *DSP* cardiomyopathy. In particular, we found that the standard DCM LVEF threshold of <35% was an insensitive marker for future severe ventricular arrhythmias in *DSP* cardiomyopathy, with many events occurring in an ejection fraction range of 35% to 55%, and occasionally at an ejection fraction >55%. We also found that criteria predictive of events in ARVC, including RV systolic dysfunction, T wave inversions,[31] and male sex[44] had no basis at all for either clinical recognition or risk stratification in *DSP* cardiomyopathy.

Our study provides compelling evidence that a reduction in LV systolic function to <55% is associated with increased risk for *DSP* cardiomyopathy, and this risk is present regardless of RV involvement. This finding is in clear contrast to our *PKP2* cohort, for which RV systolic dysfunction was the strongest marker of increased risk. Our data also suggest that frequent PVCs and LV fibrosis may contribute independently to risk prediction in *DSP* cardiomyopathy, although the lack of Holter and MRI data in a large proportion of the cohort limited the power of this analysis. Substantial risk may be present even with normal LV systolic function in the case of recurrent inflammatory episodes and extensive fibrosis on MRI. This work emphasizes the need to establish multicenter *DSP* cohorts with longitudinal follow-up to further advance risk prediction in this highly morbid condition.

Spectrum of Mutations and Variable Expressivity in *DSP* Cardiomyopathy

The location of individual truncating *DSP* mutations was relatively evenly distributed throughout the *DSP* coding sequence, and no clear correlation between truncating mutation location and clinical presentation was evident (Figure 1). These findings indicate a similar loss of function consequence of *DSP* truncating mutations through nonsense mediated RNA decay of mutant transcripts, rather than effects from truncated proteins, consistent with reduced *DSP* protein levels in skin and myocardium from patients with *DSP* truncating mutations.[18,30] The specific mutation location may contribute to the disease phenotype in the case of missense mutations (eg, mutations in the desmin versus plakophilin/plakoglobin binding domains), but this study did not include enough missense mutation carriers to analyze this relationship. Supporting this concept, a previous case series of 10 patients with missense mutations versus 17 patients with truncating mutations found that the former were less likely to have LV dysfunction.[28] Also suggesting the possibility of locus-specific effects for missense variants is the evidence of mutation clustering in the plakophilin/plakoglobin-binding and desmin-binding protein domains, as shown in Figure 1.

Despite most patients with *DSP* cardiomyopathy exhibiting a primary LDAC phenotype, an important subset (14%) exhibited RV-predominant cardiomyopathy. It is not clear from our study why this subset of individuals appeared to have a greater proclivity toward RV remodeling. Because most mutations in this study were truncating, with a presumed similar biological effect, we speculate that other genetic variation or environmental factors influenced the clinical expression. This concept is supported by the fact that different individuals within the same families experienced either LV or RV predominant cardiomyopathy.

Limitations

As a retrospective multicenter study, complete clinical data were not available for all individuals. Because of the variable penetrance of genetic cardiomyopathy, selection bias may have influenced the number of asymptomatic family members versus clinically affected family members included, potentially leading to some level of error in estimation of penetrance. In addition, lack of Holter monitors and MRIs in many of the individuals without echocardiographic evidence of cardiomyopathy may have led to underestimation of penetrance. The mean age of 36 years for patients with *DSP* and 39 for patients with *PKP2* at time of evaluation also limited our capacity to assess lifetime penetrance and outcomes. Although we establish inflammatory episodes as an important phenomenon in many patients with

DSP mutations, variable patterns in clinical practice and patient reporting likely led to underestimation of the true prevalence. This cohort was primarily composed of patients with *DSP* and *PKP2* with truncating mutations. It is possible that a subset of individuals with missense mutations could exhibit different disease features. However, the lack of pathogenic missense mutations in the experience of this multicenter cohort suggests that pathogenic missense *DSP* mutations are rare.

Conclusions

DSP mutations cause a distinct form of arrhythmogenic cardiomyopathy characterized by episodic myocardial inflammation, fibrosis, and LV systolic dysfunction that predisposes to a high rate of ventricular arrhythmias. Accurate identification, diagnosis, and risk stratification in *DSP* cardiomyopathy require a genotype-specific approach. As with the case of arrhythmogenic forms of cardiomyopathy caused by mutations in the genes *RBM20*[46] and *LMNA*,[47,48] this work supports the concept that gene-centric strategies will be essential to furthering accurate risk assessment in the age of genetic-based personalized medicine.

ARTICLE INFORMATION

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Supplemental Materials

Expanded Methods and Materials
Data Supplement Figures I–III
Data Supplement Tables I–V

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