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ORIGINAL ARTICLE

Variant Location Is a Novel Risk Factor for Individuals With Arrhythmogenic Cardiomyopathy Due to a Desmoplakin (DSP) Truncating Variant

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BACKGROUND: Truncating variants in desmoplakin (DSPv) are an important cause of arrhythmogenic cardiomyopathy; however the genetic architecture and genotype-specific risk factors are incompletely understood. We evaluated phenotype, risk factors for ventricular arrhythmias, and underlying genetics of DSPv cardiomyopathy.

METHODS: Individuals with DSPv and any cardiac phenotype, and their gene-positive family members were included from multiple international centers. Clinical data and family history information were collected. Event-free survival from ventricular arrhythmia was assessed. Variant location was compared between cases and controls, and literature review of reported DSPv performed.

RESULTS: There were 98 probands and 72 family members (mean age at diagnosis 43±8 years, 59% women) with a DSPv, of which 146 were considered clinically affected. Ventricular arrhythmia (sudden cardiac arrest, sustained ventricular tachycardia, appropriate implantable cardioverter defibrillator therapy) occurred in 56 (33%) individuals. DSPv location and proband status were independent risk factors for ventricular arrhythmia. Further, gene region was important with variants in cases (cohort n=98; Clinvar n=167) more likely to occur in the regions resulting in nonsense mediated decay of both major DSP isoforms, compared with n=124 genome aggregation database control variants (148 [83.6%] versus 29 [16.4%]; P<0.0001).

CONCLUSIONS: In the largest series of individuals with DSPv, we demonstrate that variant location is a novel risk factor for ventricular arrhythmia, can inform variant interpretation, and provide critical insights to allow for precision-based clinical management.

Key Words: cardiomyopathies, primary death, sudden cardiac desmoplakins genetic testing

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Desmoplakin is a plakin family protein that anchors the desmosome to intermediate filaments and is abundant in tissues with greater mechanical stress such as the epidermis and myocardium. Genetic variants in the gene encoding desmoplakin (DSP) cause a range of cardio-cutaneous phenotypes, including arrhythmogenic cardiomyopathy (ACM), striate palmoplantar keratoderma, and lethal acantholytic epidermolysis bullosa in more severe cases. Truncating variants (DSPtv) that lead to putative loss of function via haploinsufficiency of the protein have been previously reported as causative of disease. DSP-null mice show extensive disruption of the cytoarchitecture and cell resilience in skin and heart tissue, with death in early development.

Arrhythmogenic right ventricular cardiomyopathy (ARVC), the right dominant sub-form of ACM, is characterized by progressive loss and fibrofatty replacement of the ventricular myocardium. Diagnosis of ARVC can be challenging and 2010 Task Force Criteria consider electrical, structural (imaging and histological), and genetic characteristics. Historically, clinical descriptions of DSPtv were often based on ARVC cohorts, though growing recognition of left ventricular (LV) involvement has necessitated a shift to a broader phenotype description, ACM, encompassing left dominant arrhythmogenic cardiomyopathy and biventricular disease, with new Padua criteria proposed. Dilated cardiomyopathy and left dominant arrhythmogenic cardiomyopathy lie on a spectrum, with overlap in molecular causes. More recently, DSP has been definitely associated with both ARVC and Dilated cardiomyopathy by international gene curation expert panels. In one of the largest studies to date, clinical characteristics of DSP variants in a population of 44 probands and 63 family members were reported as a distinct ACM characterized by LV fibrosis, myocardial inflammation and high incidence of ventricular arrhythmias. Biallelic DSP variants can give rise to Carvajal syndrome, characterized by woolly hair, palmoplantar keratoderma and development of ACM in childhood, and often due to homozygous or compound heterozygous DSPtv affecting the C-terminus.

The N-terminal globular head of DSP is important in desmosome organization by binding plaque proteins such as plakophilin and plakoglobin, while the central rod domain contains a coiled-coil region. The C-terminal domain contains 3 plakin repeat domains, required for self-association and formation of homo-dimers.

Here we report an international cohort of individuals with a DSPtv. We describe the phenotype spectrum of DSPtv cardiomyopathy, family history characteristics, and provide insights into the genetic architecture of DSPtv cardiomyopathy and its relation to clinical phenotype.

**METHODS**

Data are available by request to the corresponding author and adhering to site ethical approval. All aspects of the study were performed according to institutional human research ethics committee approval according to the local sites. Institutional ethics approval was granted by individual sites; including Sydney Local Health District, Royal Prince Alfred Hospital, Australia; Royal Brompton & Harefield Hospitals Cardiovascular Biobank (National Research Ethics Service, UK). Waiver of consent was granted at Stanford School of Medicine Internal Review Board, USA. All individual-level data were de-identified.

Detailed methods are available in the Supplemental Material.

**RESULTS**

**Study Population**

Overall there were 98 probands (mean age at diagnosis 42±18 years; 59% women) and 72 family members identified (mean age at diagnosis 45±19 years; 61% women; Table 1). There were 95 probands with a cardiomyopathy and 3 with a primary cutaneous phenotype. Among family members, 48 of 72 were deemed affected, including 5 with a predominantly cutaneous phenotype. In total, 146 individuals were considered affected, including cardiomyopathy, ventricular arrhythmia and cutaneous phenotypes.

**Sex Differences**

Women were over-represented compared with men among affected individuals (86 [59%] versus 60 [41%]; Table 1). There was no difference in mean age at

**Nonstandard Abbreviations and Acronyms**

| Acronym | Definition |
|---------|------------|
| ACM     | Arrhythmogenic cardiomyopathy |
| ACMG/AMP| American College of Medical Genetics and Genomics and Association for Molecular Pathology |
| ARVC    | Arrhythmogenic right ventricular cardiomyopathy |
| CMR     | Cardiac magnetic resonance imaging |
| DSP     | Desmoplakin |
| DSPtv   | Desmoplakin truncating variant |
| HCM     | Hypertrophic cardiomyopathy |
| LGE     | Late gadolinium enhancement |
| LV      | Left ventricular |
| NMD     | Nonsense mediated decay |
| SCD     | Sudden cardiac death |

**Nonstandard Abbreviations and Acronyms**

Table 1. There was no difference in mean age at
diagnosis between women and men (40±17 years versus 46±19 years; \(P=0.07\)). Myocarditis was more frequent in men (2/43 [5%] versus 6/25 [24%]; \(P=0.046\)) but this was not always reliably reported. Women had reduced LV ejection fraction on transthoracic echocardiography, but not cardiac magnetic resonance imaging (CMR) derived LV ejection fraction. Men had greater indexed right ventricular end diastolic volume (84±20 versus 100±27; \(P=0.01\)). No differences in clinical outcomes were reported between sexes. There was a comparable distribution of variants by gene region for men and women, as well as probands and affected relatives.

### Electrophysiological Characteristics

There was a high rate of ventricular arrhythmia occurring in 56 (33%) individuals, including 46 (47%) probands and 10 (14%) family members. Ventricular arrhythmia included sudden cardiac death (SCD) in 13 (8%); 10 probands), resuscitated cardiac arrest in 15 (9%; 12 probands), appropriate implantable cardioverter defibrillator therapy in 16 (10%; 16 probands), and sustained ventricular tachycardia in 19 patients (11%; 15 probands); including 10 (14%) family members, and with some experiencing multiple events. Six probands experienced

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**Table 1. Clinical Characteristics by Proband Status and Sex**

| Diagnosis | Total, n=178 | Proband status, n=98 | Family members, n=72 | Sex (affected only) |
|-----------|--------------|----------------------|----------------------|---------------------|
|           | Affected     |                  |                      |                     |
|           | 146          | 98 (100)           | 48 (67)              | 86 (100)            |
| Mean age at diagnosis±SD, y | 43±18        | 42±18               | 45±19                | 40±17               |
| Female sex | 101 (59)     | 57 (58)             | 44 (61)              | NA                  |
| T wave inversion beyond V3 | 33 (24)      | 25 (32)             | 8 (13)               | 22 (33)             |
| Low voltages | 47 (35)      | 30 (39)             | 17 (29)              | 28 (42)             |
| Premature ventricular contractions | 35 (25)      | 23 (29)             | 12 (20)              | 25 (35)             |
| Ventricular arrhythmia | 56 (33)      | 46 (47)             | 10 (14)              | 37 (44)             |
| Sudden cardiac death | 13 (8)       | 10 (10)             | 3 (4)                | 7 (8)               |
| Resuscitated cardiac arrest | 15 (9)       | 12 (12)             | 3 (20)               | 8 (10)              |
| Sustained VT | 19 (11)      | 15 (15)             | 4 (6)                | 14 (17)             |
| Appropriate ICD therapy | 16 (10)      | 16 (16)             | 0 (0)                | 13 (15)             |
| End-stage heart failure | 10 (8)       | 7 (8)                | 3 (5)                | 6 (8)               |
| Heart transplant/LVAD | 6 (4)        | 6 (6)                | 0 (0)                | 4 (5)               |
| Combined end point | 64 (38)      | 52 (53)             | 12 (17)              | 41 (48)             |
| Myocarditis | 8 (10)       | 6 (13)              | 2 (6)                | 2 (5)               |

**Transthoracic echocardiogram**

| LV ejection fraction | 40±16 | 35±15 | 48±13 | 35±14 | 42±15 | 0.03 |
| LV end diastolic diameter | 57±9 | 59±9 | 53±8 | 58±8 | 59±10 | 0.56 |
| LV end systolic diameter | 44±12 | 47±12 | 37±9 | 47±12 | 44±12 | 0.29 |

**CMR imaging**

| LV ejection fraction, % | 42±12 | 38±12 | 48±12 | 39±11 | 43±13 | 0.14 |
| LV end diastolic volume, indexed | 111±33 | 117±35 | 100±23 | 119±34 | 105±29 | 0.09 |
| LV end systolic volume, indexed | 67±32 | 73±35 | 54±20 | 74±34 | 60±27 | 0.07 |
| RV ejection fraction, % | 47±12 | 44±12 | 53±8 | 47±11 | 45±12 | 0.46 |
| RV end diastolic volume, indexed | 89±24 | 93±25 | 83±21 | 84±20 | 100±27 | 0.01 |
| RV end systolic volume, indexed | 48±19 | 52±19 | 41±16 | 46±17 | 56±20 | 0.05 |
| Regional wall motion abnormalities | 44 (45) | 35 (56) | 9 (28) | 25 (46) | 19 (51) | 0.64 |
| Late gadolinium enhancement | 59 (61) | 40 (53) | 19 (58) | 31 (57) | 28 (76) | 0.07 |
| LV noncompaction | 22 (22) | 17 (27) | 5 (13) | 14 (29) | 8 (21) | 0.42 |

**Gene region**

| Constitutive NMD competent | 83 (57) | 57 (58) | 26 (54) | 49 (57) | 34 (57) | 0.84 |
| Nonconstitutive NMD competent | 27 (18) | 20 (20) | 7 (15) | 17 (20) | 10 (17) | 0.64 |
| Constitutive NMD incompetent | 36 (25) | 21 (21) | 15 (31) | 20 (23) | 16 (27) | 0.64 |

Data were analyzed using student t test or \(\chi^2\) test for continuous and categorical variables, respectively. Data shown are n (%) or mean±standard deviation. CMR indicates cardiac magnetic resonance; ICD, implantable cardioverter defibrillator; LV, left ventricular; LVAD, left ventricular assist device; RV, right ventricle; and VT, ventricular tachycardia.

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2 ventricular arrhythmia episodes, initially having sustained ventricular tachycardia (n=4) or resuscitated cardiac arrest (n=2), followed by appropriate implantable cardioverter defibrillator therapy. SCD or resuscitated cardiac arrest was the presenting symptom in 24 (14%; 20 probands) patients. T wave inversion beyond V3 occurred in 33 (24%), low voltages in 47 (35%) and premature ventricular contractions in 56 (33%).

Imaging Characteristics

Echocardiographic and CMR characteristics are shown in Table 1. Signs of LV noncompaction were reported (n=22), with 6 having a ratio of noncompacted to compacted layer >2.3 on CMR. Four probands were reported to have hypertrophic cardiomyopathy (HCM) with ages at diagnosis ranging from 58 to 83 years, and LV hypertrophy measuring 26 mm, 16 mm, and an apical pattern in 2. DSPtv are not established as associated with HCM, and we consider it unlikely that these variants are causal for HCM for these 4 cases, but are reported as they met the pre-specified eligibility criteria. Late gadolinium enhancement (LGE) was reported in 59 (61%), and end-stage heart failure was reported in 10 (8%) patients. Two women developed disease while pregnant, 1 showed impaired LV function (LV ejection fraction <45%) at 32 weeks of gestation, while the other developed narrow complex tachycardia at 38 weeks of gestation with subsequent echocardiogram showing a dilated and impaired LV. A further 2 women developed disease during the postpartum period. Finally, another patient who died suddenly during pregnancy was identified to be positive for Parvovirus B19 on postmortem Parvo-polymerase chain reaction in myocardial tissue. Myocarditis was reported in 7 individuals on CMR (and another on postmortem investigation).

Genetic Analysis

A total of 69 distinct DSPtv were identified in the 98 probands (Table S1). Among the 69 DSPtv, there were 31 small insertions or deletions leading to a frameshift and downstream premature termination codon, 25 nonsense variants, 12 canonical splice-site altering variants and a large deletion of exons 5–24. Eleven (16%) variants were classified as pathogenic, 57 (83%) were classified as likely pathogenic and 1 (1%) was classified as a variant of uncertain significance. Two probands had a diagnosis of cardiomyopathy, with woolly hair and keratoderma (OMIM 605676) and were compound heterozygous, each carrying a DSPtv (p.Arg2229Serfs*32 or p.Tyr28Alafs*66) and a DSP splice site variant (the same c.273+5G>A in both). This splice site variant has or p.Tyr28Alafs*66) and a splice site variant (the

Event-Free Survival From Ventricular Arrhythmia Based on Gene Region

Information with regard to occurrence of ventricular arrhythmia or censoring was available for 167 individuals. There were 56 probands and family members who experienced a ventricular arrhythmia during their lifetime. Univariable Cox proportional hazards models showed gene region and proband status as significantly associated with worse survival from ventricular arrhythmias (Table 3; Figure 2). Adjusting for other variables, variants in the constitutive NMD competent region (HR, 2.8 [95% CI, 1.3–6.0]; P=0.01), nonconstitutive NMD-competent region (HR, 3.2 [95% CI 1.3–7.9]; P=0.009) and proband status (HR, 3.3 [95% CI, 1.7–6.6]; P=0.0006)
remained significant independent life-time risk factors for ventricular arrhythmia (Table 3).

**Cutaneous Phenotype**

Cutaneous abnormalities were not systematically reported; however, notably 1 family with a DSPtv in the nonconstitutive NMD-competent region (cardiac isoform, DSPI) had an affected relative with hyperkeratosis and cardiomyopathy. An additional 13 individuals with DSPtv in the constitutive NMD-competent region and 5 in the constitutive NMD-incompetent region were reported with overt cardio-cutaneous features noted at clinical review. In 8 patients (5%; 3 probands) only cutaneous abnormalities were reported, and were the sole finding in 1 family following an autosomal dominant inheritance pattern.

**Postmortem Findings and Cardiac Transplant Histology**

Thirteen patients (8%; 10 probands) presented with SCD (Table S4). In all 13, a postmortem investigation was performed. The mean age at death was 26±11 years. Where recorded, the activity at time of death varied from exercise through to sleep. No decedent had a pre-morbid diagnosis of a cardiac condition. Nine decedents received a postmortem diagnosis of ARVC or probable ARVC. There was LV involvement in all cases and fibrosis and fatty infiltration commonly reported.

Two patients underwent a heart transplant due to end stage heart failure. Biventricular involvement was observed in both hearts, as were signs of LV noncompaction. One heart showed ARVC with septal involvement and replacement fibrosis in both ventricles and septum. The other heart showed LV noncompaction with notable right ventricular involvement consisting of fatty changes and atrophy.

**Family History Characteristics**

Among the probands, 49 (51%) had a documented family history of cardiomyopathy, while 16 (17%) had a family history of a suspicious SCD under the age of 40 years. Of the 72 family members with positive gene results included, 48 (67%) had overt disease, while 24 (33%) remained asymptomatic (mean age of 49±22 years and 15 [63%] were women). There were 8 family members aged 60 years or older (60–86 years; 5 women) with no clinical evidence of disease, suggesting incomplete penetrance. By gene region, there was no statistical difference in the proportion of probands with a positive

**Figure 1. Linear topology schematic showing distribution of DSPtv across key gene regions of major isoforms DSPi and DSPii.**

Case variants (Cohort, Clinvar, and Literature) are shown above and control variants below the line. The number of unique variants (per proband) from each source are shown in the table. CR indicates central fibrous rod domain; DSPtv, desmoplakin truncating variant; G1, globular 1; G2, globular 2; gnomAD, gnome aggregation database; and NMD, nonsense mediated decay.
family history (constitutive NMD-competent 27 [49%], nonconstitutive NMD-competent 13 [65%], constitutive NMD-incompetent 9 [43%], \( P=0.33 \)).

**Table 2. Cardiac Investigation of Affected Individuals With DSPtv by Gene Region**

|                         | \( N \) | Constitutive NMD-competent | Nonconstitutive NMD-competent | Constitutive NMD-incompetent | \( P \) value |
|-------------------------|---------|----------------------------|-------------------------------|-------------------------------|--------------|
| \( n \)                 | 146     | 83 (57)                    | 36 (25)                      | 27 (18)                      | –            |
| Age at diagnosis, y     | 143     | 40±18                      | 37±16                        | 52±16                        | 0.002        |
| Female sex              | 146     | 49 (59)                    | 17 (63)                      | 20 (56)                      | 0.84         |
| Family history of disease | 107     | 36 (57)                    | 15 (68)                      | 9 (43)                       | 0.25         |
| Ventricular arrhythmia  | 144     | 34 (41)                    | 14 (54)                      | 8 (22)                       | 0.03         |
| SCD                     | 143     | 7 (9)                      | 4 (15)                       | 2 (6)                        | 0.40         |
| Resuscitated cardiac arrest | 143   | 7 (9)                      | 5 (23)                       | 2 (6)                        | 0.06         |
| Sustained VT            | 143     | 14 (17)                    | 1 (4)                        | 4 (11)                       | 0.19         |
| Appropriate ICD therapy | 143     | 13 (16)                    | 3 (12)                       | 0 (0)                        | 0.04         |
| Combined end point      | 144     | 39 (48)                    | 15 (58)                      | 10 (28)                      | 0.04         |
| Myocarditis             | 68      | 6 (18)                     | 1 (5)                        | 1 (7)                        | 0.32         |
| LV noncompaction        | 87      | 14 (26)                    | 6 (40)                       | 2 (11)                       | 0.16         |
| PVCs                    | 119     | 21 (33)                    | 4 (18)                       | 10 (30)                      | 0.43         |

**Translational echocardiogram**

|                         | \( n \) | \( LVEF \) | \( LVEDD \) | \( LVESD \) | \( P \) value |
|-------------------------|---------|------------|------------|------------|--------------|
| Ejection fraction       | 100     | 41±16      | 28±11      | 38±13      | 0.008        |
| LV end diastolic diameter | 98      | 59±9       | 57±10      | 57±7       | 0.71         |
| LV end systolic diameter | 77      | 45±12      | 47±14      | 46±9       | 0.95         |

**CMR imaging**

|                         | \( n \) | \( LVEF \) | \( LVEDV \) | \( LVESV \) | \( RV EF \) | \( RV EDV \) | \( RV ESV \) | \( Late Gadolinium Enhancement \) | \( Regional Wall Motion Abnormalities \) | \( Electrocardiogram \) |
|-------------------------|---------|------------|------------|------------|------------|------------|------------|----------------------------------|----------------------------------|----------------------|
| Ejection fraction       | 88      | 41±12      | 38±13      | 41±11      | 0.55       |
| LV end diastolic volume, indexed | 75 | 121±39     | 114±28     | 104±22     | 0.13       |
| LV end systolic volume, indexed | 76 | 75±37     | 69±30     | 61±24     | 0.27       |
| RV ejection fraction    | 62      | 49±10      | 45±11      | 44±14      | 0.35       |
| RV end diastolic volume, indexed | 56 | 92±26     | 81±14     | 93±25     | 0.35       |
| RV end systolic volume, indexed | 55 | 51±19     | 48±13     | 51±21     | 0.87       |
| Late gadolinium enhancement | 91 | 29 (64) | 12 (63) | 18 (67) | 0.97 |
| Regional wall motion abnormalities | 91 | 22 (47) | 6 (35) | 16 (59) | 0.29 |

**Sinus rhythm**

|                         | \( n \) | \( PR \) interval | \( QRS \) | \( QTc \) | \( T wave inversion beyond V3 \) | \( Low voltages \) | \( P \) value |
|-------------------------|---------|-------------------|-----------|----------|---------------------------------|-------------------|--------------|
| Sinus rhythm            | 81      | 43 (98)           | 16 (100)  | 21 (100) | 0.65                            | 0.53               |
| PR interval             | 110     | 167±43            | 155±27    | 172±50   | 0.35                            | 0.35               |
| QRS                     | 108     | 102±17            | 95±12     | 97±16    | 0.21                            | 0.21               |
| QTc                     | 105     | 424±40            | 417±32    | 421±39   | 0.76                            | 0.76               |
| T wave inversion beyond V3 | 117 | 21 (33)           | 3 (13)    | 7 (23)   | 0.14                            | 0.14               |
| Low voltages            | 114     | 23 (37)           | 8 (38)    | 10 (33)  | 0.93                            | 0.93               |

Data shown are mean±standard deviation (\( n \) = number of persons with available data) or \( n \) (%). ICD indicates implantable cardioverter defibrillator; LV, left ventricle; NMD, nonsense mediated decay; PVCs, premature ventricular contractions (>500 per 24 hours); RV, right ventricle; and SCD, sudden cardiac death.

Literature Review of Previously Reported DSPtv

Three hundred and fifteen studies were identified, 240 were screened, and 185 full texts were assessed for eligibility (85 were excluded from the final qualitative synthesis, including 66 that did not report any DSP variant, 2 where phenotype was not provided, 2 with no full-text article available, and 1 review; Figure S1). Of the 98 studies (describing both disease and genotype-first cohorts) included in the final selection, a total of 105 DSPtv in 143 probands from apparently unrelated families were reported, including 57 nonsense, 42 frameshift, and 6 splice site variants (Tables S5–S7). All reported variants were absent or very rare (allele count \( \leq 2 \)) in gnomAD and were classified as pathogenic or likely pathogenic. One variant (p.Thr2104fs*12) was present 13 times in gnomAD, however has strong evidence of pathogenicity and reported in a compound heterozygous state.

Both dominant and recessive patterns of inheritance of DSPtv were reported. Cascade genetic testing to confirm autosomal dominant inheritance was reported for only 19 DSPtv (dominant DSPtv) in 22...
families. Families reported with autosomal dominant inheritance commonly demonstrated adult age of onset, incomplete penetrance and variable clinical expression. Of the 105 reported DSPtv, 26 were only identified in affected individuals with homozygous or compound heterozygous inheritance. Four DSPtv co-occurred in trans with one of 3 missense DSP variants (p.Ala2655Asp, p.Arg2366Cys, and p.Asn287Lys), each of which involved highly conserved residues within globular heads, are absent in gnomAD, and classified as likely pathogenic. There were 16 individuals with 23 DSPtv identified to have autosomal recessive disease, either homozygous (n=9) or compound heterozygous (n=7). In just those variants identified in a homozygous state there was only 1 (11%) in the constitutive NMD-competent region, 5 (56%) in the nonconstitutive NMD-competent region, and 3 (33%) in the constitutive NMD-incompetent region.

**DISCUSSION**

DSPtv lead to a distinct cardiomyopathy characterized by LV involvement and a high-risk of ventricular arrhythmia and SCD. We present a large international series of cases with DSPtv and demonstrate that the location of the DSPtv is a novel risk factor for ventricular arrhythmia (Figure 3). Truncating variants in the constitutive NMD-competent region were enriched in cases compared with controls, and predicted to result in NMD and haploinsufficiency of both DSPI and DSPII. Our findings highlight the importance of personalized medicine and the move towards gene-guided management of patients in the future.

Ventricular arrhythmias occur frequently in patients with DSP cardiomyopathy, with one previous study reporting 23% presenting with SCD events.18 In our cohort, 47% of probands had ventricular arrhythmia either at presentation or during follow-up. In addition, 14% of relatives experienced ventricular arrhythmia, including 7% as their initial presenting symptom. This included 2 probands who presented with resuscitated cardiac arrest without any overt structural abnormalities of the heart, supporting the notion that life-threatening electrical phenotype can precede overt cardiac structural disease.19–21 While we were unable to robustly ascertain clinical risk factors due to the large proportion of cases who presented with ventricular arrhythmia (ie, without necessary pre-event clinical data), a recent series of 107 patients with any DSP variants (n=30 events) showed ventricular arrhythmias were associated with reduced LV ejection fraction, while premature ventricular contractions (>500 beats in 24 hours), LGE, and right ventricular dysfunction were not shown to be associated with ventricular arrhythmia.12 Family history of SCD has not previously been evaluated in this group, and we showed it is not associated with ventricular arrhythmia in our population.

Prior observation that DSPtv are predominantly associated with a left dominant form of ACM25,22 is in line with our findings. Recent examples of DSPtv presenting as recurrent myocarditis and acute myocardial infarction-like events have also been reported.23,24 Women were overrepresented in our population, but otherwise shared similar clinical characteristics compared with men, except reduced indexed right ventricular end diastolic diameter on CMR. This finding is in contrast to other reported inherited cardiomyopathy patient cohorts, where a higher prevalence of men is often reported.25–27 Of note, a recent report of ARVC presenting as clinical myocarditis showed low QRS voltage and negative T waves beyond V3. Low QRS voltage in limb leads have previously been shown to be associated with ventricular arrhythmia.28 DSPtv cardiomyopathy patients frequently had low QRS voltage and negative T waves beyond V3. Low QRS voltage in limb leads have previously been shown to be associated with ventricular arrhythmia.28,29 Four probands were reported to have HCM, however it should be noted that all 4 probands were men, presenting in older age and 3 had mild LV hypertrophy, all

| Table 3. Lifetime Risk Factors for Ventricular Arrhythmia for Individuals With a DSPtv |
|---------------------------------|-----|---------|-------|---------|-----|---------|
|                                | N   | HR      | 95% CI | P value | N   | HR      | 95% CI | P value |
| Female sex                     | 167 | 1.5     | 0.9–2.6| 0.14    |     |         |        |         |
| Proband status                 | 167 | 3.5     | 1.8–7.0| 0.0003  |     | 3.3     | 1.7–6.6| 0.0006  |
| Gene region:                   |     |         |        |         |     |         |        |         |
| Constitutive NMD competent     | 2.8 | 1.3–6.1 | 0.009  | 2.8     | 1.3–6.0| 0.01    |
| Non-constitutive NMD competent | 3.8 | 1.6–9.2 | 0.003  | 3.2     | 1.3–7.9| 0.009   |
| Constitutive NMD incompetent   |     |         |        |         |     |         |        |         |
| Family history of sudden cardiac death | 110 | 0.7     | 0.3–1.7| 0.44    |     |         |        |         |

For the adjusted analysis, there were total n=167 individuals included with n=56 events. DSPtv indicates desmoplakin truncating variant; NMD, nonsense mediated decay; and REF, reference category.
characteristics previously described in the nonfamilial sub-group of HCM. Previous assessment of the clinical validity of DSP variants causing HCM failed to identify sufficient evidence of gene-disease association. While our finding remains unclear, it seems reasonable to consider these clinical diagnoses as unrelated to the DSP variants.

A recent systematic evaluation of cutaneous abnormalities among DSP variants showed all patients expressed some degree of skin or hair abnormalities, except those with DSP variants in the nonconstitutive NMD-competent region (cardiac isoform, DSPI). Interestingly, we report 1 proband and their affected relative with palmoplantar keratoderma, with a DSP variant in the nonconstitutive NMD-competent region. Another study reported 10% of DSP variants had cutaneous disease only, while 12% were reported to have LV dominant ACM and cutaneous disease.

We show DSP variants localized to the constitutive NMD-competent region, corresponding to the N-terminal globular head, were enriched in patients compared with controls, and this finding was replicated in the variants identified through literature review. This region plays a critical role in organization and assembly of the desmosomal complex by binding with plakophilin and plakoglobin. One previous report of DSP missense variants in patients with a clinical diagnosis of ARVC suggested a potential

![Figure 2. Independent lifetime risk factor for ventricular arrhythmia.](http://ahajournals.org)

(A) Gene region including probands only. (B) Gene region including probands and affected family members. Time is given in years. NMD indicates nonsense mediated decay.

![Figure 3. Summary of the key findings and illustration of the impact of DSP variants location on protein expression (for DSPI).](http://ahajournals.org)

DSC indicates desmocollin-2; DSG2, desmoglein-2; JUP, plakoglobin; PKP2, plakophilin-2; and NMD, nonsense mediated decay.
“hotspot” N-terminal region, with 8/17 (47%) missense variants localized to the N-terminal compared with 1/28 (4%) of controls (P<0.0008). Further, they concluded DSPtv were significantly more prevalent in ARVC cases than controls. Indeed, a recent study also showed clustering of missense variants in the N-terminal, but reported DSPtv to be more evenly distributed across the gene,12 potentially limited by sample size. Another showed enrichment of missense variants in the spectrin repeat domain, which is part of the constitutive NMD competent region.34
While it seems likely that truncating variants in the NMD-incompetent region escape NMD and have a later onset and less deleterious impact, functional work to date has shown highly variable pattern of protein expression representing both haploinsufficiency and dominant negative effects.25 Our literature review identified biallelic DSPtv localized more often to the constitutive NMD-incompetent region compared with dominant DSPtv, suggesting that single heterozygous DSPtv are more likely to cause disease when occurring in the NMD-competent regions. Further, very few cases with homozygous variants in the constitutive NMD-competent region have been reported, with 1 example of a sib pair with severe lethal acantholytic epidermolysis bullosa, who died at 1 and 3 days respectively.36 It seems unlikely these infants were DSP null, given DSP knockout mice show embryonic lethality,4 suggesting expression of low-level truncated protein may be able to rescue the phenotype to some degree. Taken together, identification of a DSPtv in the NMD-competent regions should be considered important and may prompt gene and disease-specific adaptation and use of the ACMG/AMP (American College of Medical Genetics and Genomics and Association for Molecular Pathology) criteria.37 We suggest DSPtv in this region be allocated very strong level of evidence, PVS1, when considering pathogenicity, when seen in an individual with a well characterized and concordant phenotype.

STUDY LIMITATIONS
This was a large retrospective cohort study, and while it was an international effort, differences in practices and data collection by site meant some variables were incomplete. Furthermore, the event rate and data missingness precluded more detailed risk factor analyses. Diagnosis was made by referring clinician and most recruitment was from specialized tertiary referral centers and therefore likely represents more severe phenotypes. The literature review was limited by publication bias, and inconsistent reporting of clinical, family and genetic information.

CONCLUSIONS
We present a large international series of individuals with DSPtv and show gene region is a novel risk factor, specifically DSPtv leading to predicted NMD of truncated protein and haploinsufficiency of DSPI and/or DSPII is a risk factor for ventricular arrhythmias. By sub-typing disease by genotype there is increasing ability to offer precision medicine-based advice and therapies, and thereby improved outcomes for patients and their families.

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desmoplakin I and II mutations underlie dominant arrhythmogenic cardiomyopathy with a hair and skin phenotype. Br J Dermatol. 2019;180:1114–1122. doi: 10.1111/bjd.17388

33. Kapplinger JD, Landstrom AP, Salisbury BA, Callis TE, Pollevick GD, Tester DJ, Cox MG, Bhuiyan Z, Bikker H, Wiesfeld AC, et al. Distinguishing arrhythmogenic right ventricular cardiomyopathy/dysplasia-associated mutations from background genetic noise. J Am Coll Cardiol. 2011;57:2317–2327. doi: 10.1016/j.jacc.2010.12.036

34. Grondin S, Wazirián AC, Jorda P, Terrone DG, Gagnon J, Robb L, Amyot J, Rivard L, Page S, Talajic M, et al. Missense variants in the spectrin repeat domain of DSP are associated with arrhythmogenic cardiomyopathy: a family report and systematic review. Am J Med Genet A. 2020;182:2359–2368. doi: 10.1002/ajmg.a.61799

35. Rasmussen TB, Hansen J, Nissen PH, Palmfeldt J, Dalager S, Jensen UB, Kim WY, Heikkendorff L, Mølgaard H, Jensen HK, et al. Protein expression studies of desmoplakin mutations in cardiomyopathy patients reveal different molecular disease mechanisms. Clin Genet. 2013;84:20–30. doi: 10.1111/cge.12056

36. Bolling MC, Veenstra MJ, Jonkman MF, Diercks GF, Curry CJ, Fisher J, Pas HH, Bruckner AL. Lethal acantholytic epidermolysis bullosa due to a novel homozygous deletion in DSP: expanding the phenotype and implications for desmoplakin function in skin and heart. Br J Dermatol. 2010;162:1388–1394. doi: 10.1111/j.1365-2133.2010.09668.x

37. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–424. doi: 10.1038/gim.2015.30

38. Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, Gu B, Hart J, Hoffman D, Hoover J, et al. ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res. 2016;44:D862–D868. doi: 10.1093/nar/gkv1222

39. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfoldi J, Wang Q, Collins R, Lariachia KM, Ganna A, Birnbaum DP, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020;581:434–443. doi: 10.1038/s41586-020-2308-7

40. Mazzarotto F, Tayal U, Buchan RJ, Midwinter W, Wilk A, Whiffin N, Govind R, Mazaika E, de Marvao A, Dawes TJW, et al. Reevaluating the genetic contribution of monogenic dilated cardiomyopathy. Circulation. 2020;141:387–398. doi: 10.1161/CIRCULATIONAHA.119.037661

41. Abou Tayoun AN, Pesaran T, DiStefano MT, Oza A, Rehm HL, Biesecker LG, Harrison SM; ClinGen Sequence Variant Interpretation Working Group (ClinGen SVI)ClinGen Sequence Variant Interpretation Working Group (ClinGen SVI). Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. Hum Mutat. 2018;39:1517–1524. doi: 10.1002/humu.23626

42. Lazzarini E, Jongbloed JD, Pilichou K, Thiene G, Basso C, Bikker H, Charbon B, Swertz M, van Tintelen JP, van der Zwaag PA. The ARVD/C genetic variants database: 2014 update. Hum Mutat. 2015;36:403–410. doi: 10.1002/humu.23765