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Severe cardiac phenotype with right ventricular predominance in a large cohort of patients with a single missense mutation in the *DES* gene

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BACKGROUND Desmin-related myopathy is a clinically heterogenous group of disorders encompassing myopathies, cardiomyopathies, conduction disease, and combinations of these disorders. Mutations in the gene encoding desmin (*DES*), a major intermediate filament protein, can underlie this phenotype.

OBJECTIVE The purpose of this study was to investigate the clinical and pathologic characteristics of 27 patients from five families with an identical mutation in the head domain region (p.S13F) of desmin.

METHODS/RESULTS All 27 carriers or obligate carriers of a p.S13F *DES* founder mutation demonstrated a fully penetrant yet variable phenotype. All patients demonstrated cardiac involvement characterized by high-grade AV block at young ages and important right ventricular (RV) involvement. RV predominance was demonstrated by the presence of right bundle branch block in 10 patients (sometimes as a first manifestation) and by RV heart failure in 6 patients, including 2 patients who fulfilled the diagnostic criteria for arrhythmogenic RV cardiomyopathy. Because of this clinical overlap with desmosome cardiomyopathies, we also studied the organization of the intercalated disks, particularly the distribution of desmosomal proteins. Normal amounts of the major desmosomal proteins were found, but the intercalated disks were more convoluted and elongated and had a zigzag appearance.

CONCLUSION In this largest series to date of individuals with a single head domain *DES* mutation, patients show a variable yet predominantly cardiologic phenotype characterized by conduction disease at an early age and RV involvement including right bundle branch block and/or RV tachycardias and arrhythmogenic RV cardiomyopathy phenocopies. A localized effect of desmin on the structure of the cardiac intercalated disks might contribute to disease pathogenesis.

KEYWORDS Cardiomyopathy; Genetics; Heart block; Bundle branch block

ABBREVIATIONS **ARVC** = arrhythmogenic right ventricular cardiomyopathy; **CK** = creatine phosphokinase; **Cx43** = connexin43; **DCM** = dilated cardiomyopathy; **DES** = desmin; **PCR** = polymerase chain reaction; **RBBB** = right bundle branch block; **RCM** = restrictive cardiomyopathy; **RV** = right ventricular

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Introduction

The *DES* gene encodes desmin, which is a major intermediate filament protein of skeletal and cardiac muscle that provides structural and functional integrity by coordinating mechanical stress transmission, organelle positioning, organization and assembly of sarcomeres, signal transduction,

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and apoptosis.[1] Mutations in the *DES* gene are associated with a variable clinical phenotype referred to as desmin-related myopathy (OMIM #601419). The clinical phenotype encompasses "isolated" myopathies, pure cardiac phenotypes (including dilated cardiomyopathy [DCM] and restrictive cardiomyopathy [RCM]), cardiac conduction disease, and combinations of these disorders.[2–10] If both cardiologic and neurologic features occur, they can manifest in any order, as cardiologic features can precede, occur simultaneously with, or follow manifestation of generalized neuromuscular disease.

More than 40 *DES* mutations have been identified in 53 different index patients. The majority of mutations are located in the α-helical rod domains of the gene. Potential genotype–phenotype relationships are emerging. It has recently been suggested that mutations in the 2B segment of desmin are mainly involved in skeletal muscle disease, whereas mutations in the 1B and tail domain cause more serious cardiac disease.[11]

We have identified five Dutch families, two of which have recently been partially described, with a variable yet predominantly cardiologic phenotype among individuals carrying an identical missense mutation in the head domain of the *DES* gene.[12]

The aim of this study was to investigate (1) the impressive phenotypic variability in the largest series of patients with a single *DES* mutation that is located in the head domain (p.S13F); (2) the occurrence of right-sided myocardial involvement including arrhythmogenic right ventricular cardiomyopathy (ARVC)-like phenotypes; and (3) the effect of the p.S13F mutation on cell–junction organization.

Methods

Clinical evaluation

The five index patients were referred to our cardiogenetics outpatient clinic, UMC Groningen, with either a primary cardiologic phenotype (two index patients) or a neurologic phenotype associated with cardiac manifestations (three index patients). Two of the index patients were recently described.[12] The index patients and their relatives underwent a regular clinical genetic counseling procedure. Patients who gave informed consent were evaluated for cardiologic and/or neurologic and genetic characteristics.

All probands and family members were evaluated cardiologically by 12-lead ECG, echocardiography, 24-hour Holter registration, and exercise testing. Echocardiography was performed using established techniques and following the guidelines of the American Society of Echocardiography.[13] The criteria and methods of investigation proposed by Mestroni et al[14] were used to diagnose DCM, and the generally accepted task force criteria were used to diagnose ARVC.[15]

In patients with a primary cardiologic phenotype, the initial neuromuscular examination was restricted to obtaining a patient and family case history of neuromuscular complaints, a physical examination performed by an experienced neurologist, and a creatine phosphokinase (CK) measurement. If these results were negative, no additional neurologic examinations were performed.

Mutation analysis

Genomic DNA was isolated from blood samples obtained from the five index patients and 27 relatives. DNA from 300 chromosomes from ethnically matched, healthy individuals was used as a control group. Written informed consent was obtained from all participants according to the UMCG Medical Ethics Committee.

Primers for amplification of the *DES* gene were designed to jointly encompass the protein coding sequences of exons as well as the intronic flanking regions containing sequences essential for correct splicing, as described previously.[16] The genomic sequences used to design these primers were obtained from sequences on the GenBank database (accession number NC_000002.10, region 219991343 to 219999705) and on the NCBI web site (www.ncbi.nih.gov/projects/genome/guide/human). Amplifications were conducted following a standard polymerase chain reaction (PCR) protocol, and PCR products were analyzed by denaturing gradient gel electrophoresis or direct sequencing.[17] PCR fragments that showed an aberrant denaturing gradient gel electrophoresis pattern were sequenced as described previously.[18] Our primer sequences and PCR conditions are available upon request.

Genealogy

For discovery of any distant relationships between index patients, the genealogies of the patients were investigated using data from civil registers and state archives, and the pedigrees were reconstructed to approximately AD 1800 (covering 6–8 generations).

Haplotype analysis

Fourteen microsatellite markers around *DES* were selected with the NCBI Map Viewer and analyzed for seven patients (A-VI-2, B-VI-9, B-VI-10, C-VIII-8, D-II-5, E-I-2, E-II-1; Figure 1) from five different families and 12 control persons (primers and conditions available upon request). The method described by Machado et al[19] was used to calculate the age of the mutation.

Histology, immunohistochemistry, and immunofluorescence

For three patients (A-VI-2, B-VI-9, D-II-3) and two unaffected controls, myocardial tissue was available for light microscopy and/or immunohistochemical staining. For patients D-II-3 and C-VII-10, myocardial tissue for electron microscopy was available. Tissue samples were prepared using routine procedures. Immunohistochemical staining of representative tissue sections was performed (Department of Pathology, UMC Groningen) using a monoclonal antibody reactive with desmin (1:50, clone DE-R-11, Dako, Glostrup, Denmark) and a monoclonal antibody reactive with sequestosome-1 (1:100, clone sc-28359, Santa Cruz Biotechnology, Santa Cruz, CA, USA). A Ventana Bench-

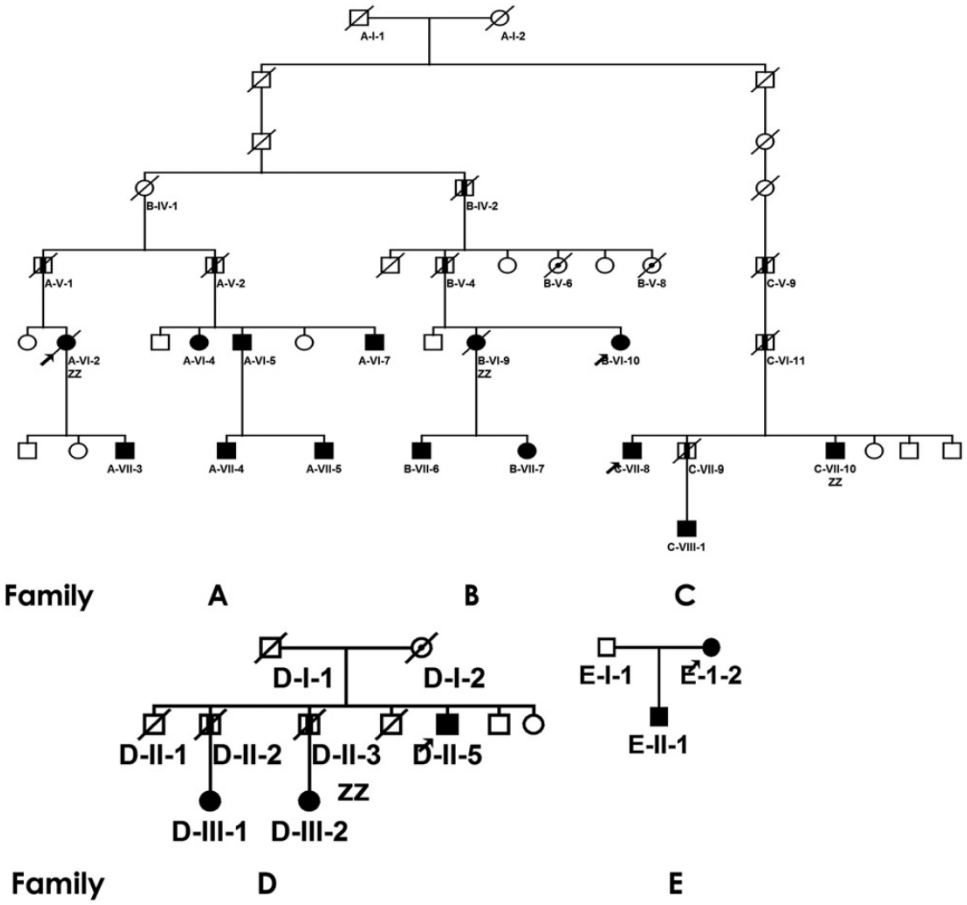

Figure 1 Pedigrees of the families studied. *Square symbols* indicate men; *circles* indicate women; *black filled symbols* indicate clinical signs and 38C>T mutation; *vertical bar in symbol* indicates obligate carrier of 38C>T mutation; *diagonal line through symbol* indicates deceased; *line within symbol* indicates signs of desminopathy but not genetically confirmed; *arrow* indicates index patient of that family. zz = zigzag intercalated disks.

mark immunostainer (Ventana Medical Systems, Tucson, AZ, USA) was used according to the manufacturer's protocol. Appropriate positive and negative controls were used.

Immunostaining of samples from the patients and two controls was performed (Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA) using primary antibodies, including mouse monoclonal anti-plakoglobin and anti-pan cadherin (Sigma, St. Louis, MO, USA), mouse monoclonal anti-desmocollin 2/3 (Zymed, Vienna, Austria), rabbit polyclonal anti-Cx43 (Sigma), rabbit polyclonal anti-desmoplakin (Serotec, Kidlington, UK), and rabbit polyclonal anti-desmin (AbCam, Cambridge, UK), and secondary antibodies cyanine 3 (Cy3) conjugate AffiniPure goat anti-mouse/anti-rabbit immunoglobulin G (Jackson Immunoresearch, West Grove, PA, USA). Immunostained preparations were analyzed by laser scanning confocal microscopy (Sarastro model 2000, Molecular Dynamics, Sunnyvale, CA, USA) as previously described.[20]

For electron microscopy (UMCG, The Netherlands), endomyocardial biopsy samples were processed by fixation in 2% buffered glutaraldehyde, stained with 1% osmium tetroxide, and embedded in Epon 812 (Serva, Heidelberg, Germany).

Results

Clinical

Phenotypic description

Detailed clinical data of all individual *DES* 38C>T mutation or obligate carrier patients and those likely to be affected, including outcomes and results of previous histology, are given in Table 1. The pedigrees are shown in Figure 1. The clinical criteria for two patients who met the criteria for ARVC are given in Table 2. Results of clinical evaluation were available for 22 individuals from the five families, and limited information was available for another five patients who were obligate mutation carriers.

From the proven mutation or obligate carriers group, sufficient data were available for 22 individuals, all of whom had a cardiac phenotype: 16 (73%) initially presented with a pure cardiac phenotype, 3 (14%) presented with cardiac symptoms preceding neurologic symptoms, and 3 patients presented with neurologic symptoms before or simultaneously with cardiac pathology.

Mean age at initial presentation of the 20 patients for whom this information was available was 35.5 years [females: 41.6 years (range 27–54 years); males: 32.2 years

Table 1 Clinical features of proven or obligate *DES* 38C>T carriers and family members likely to be mutation carriers

Patient ID	Gender	Genetic status	Referred for (age)	Cardiologic: ECG/rhythm (age)	Cardiologic: Structural (age)	Neuromuscular phenotype (age)	Pathology EMB/obduction (age)	Outcome/follow-up (age)
A-V-1	M	OC		"Cardiac disease"				Died (40)
A-V-2	M	OC		"Cardiac disease"				Died (41)
A-VI-2 (index)	F	38C>T	Palpitations	cAVB (40) PM AF (47) NSVT	DCM (54) RV>LV	Normal	EMB: aspecific abnormality/hypertrophy	Died (58) progressive RV failure
A-VI-4	F	38C>T	Screening (50)	LBBB/RBBB (50) Low-voltage (52) VPBs (52)	Left atrial size†	Mild proximal myopathy legs (52) CK normal		Hypertension Palpitations (52)
A-VI-5	M	38C>T	HF (21)	Low voltage, RBBB, AF (21)	HF RV>LV (21) TI (22) ARVC Task Force criteria+[21] (Table 2)	Mild polyneuropathy (43) CK normal	EMB (21) fibrosis Explanted heart: dilated RVOT; compatible with ARVC (Figure 2)	HTx (27) end-stage RV/LV failure Lymphoma (38); chemotherapy
A-VI-7	M	38C>T	Collapse (37)	LBBB VTs (37) Low voltage Negative T V_{2-3}	ARVC Task Force criteria+ (Table 2) DCM (37) RV+LV failure	Normal (37) CK normal		ICD (37) 2 years uneventful
A-VII-3	M	38C>T	Palpitations Exercise related (<17)	Repolarization abnormality VPBs, VTs (18) LBBB VTs[21]	Normal (28)	Normal (28) CK normal		Stable (26) ICD (28)
A-VII-4	M	38C>T	Screening (14)	Repolarization abnormality RBBB (14) VPBs (18)	RCM-like (15) LVH	NA CK normal		Palpitations Impaired exercise tolerance
A-VII-5	M	38C>T	Screening (14)	Repolarization abnormality Epsilon wave, RBBB VTs (14)	Normal (14) Mild diastolic dysfunction	Normal CK normal (15)		Collapse, ICD (15)
B-IV-2	M	OC	Collapse	RBBB (53) Tachycardias				"Cardiac disease"/myocardial infarction? (53)
B-V-4	M	OC		cAVB (47) VT, CM, PM (47)	Mild DCM (58)	Distal/bulbar weakness (58) Diabetic polyneuropathy CK NA		CAD; died HF (60)
B-V-6	F	Likely						Cardiac complaints; stayed in bed; recurrent collapse, SCD (31)
B-V-8	F	Likely	Near collapse/palpitations (47)	cAVB (47) PM (51) VT	No signs of CM	Prox LGMD-like (46)	Muscle biopsy: mild aspecific abnormality, fibrosis (46), muscular dystrophy (52)	CAD (59) Died myocardial infarction (59)
B-VI-9[12]	F	38C>T	Palpitations (±27)	cAVB/PM (±27) VT (30) AF (49)	Unspecified CM (±27) RV HF (49)	Dist>prox (36) LGMD CK ↑	EMB (51): myocyte hypertrophy/fibrosis; desmin aggregates Postmortem: RV dilatation, hypertrophic cardiomyocytes, fibrosis, desmin aggregates	Died RV HF (51)
B-VI-10[12]	F	38C>T	Palpitations VPBs (42)	VT, SVT, VPBs, LAHB (43) cAVB/PM (45)	Normal (45/47)	Prox>dist muscular weakness (30) (obj 45 years) LG distribution CK ↑ – ↑↑	Muscle biopsy: desmin accumulation	Alive (50)
B-VII-6	M	38C>T	Collapse (31)	cAVB PM (31)	Mild LVH, diastolic LV dysfunction (33)	NA		No progression (35)
B-VII-7	F	38C>T	Collapse (35)	cAVB PM (37)	Mild diastolic LV dysfunction (37)	NA CK ↑		
C-VI-11	M	OC						Died in unexplained accident (46)

Table 1 Continued

Patient ID	Gender	Genetic status	Referred for (age)	Cardiologic: ECG/rhythm (age)	Cardiologic: Structural (age)	Neuromuscular phenotype (age)	Pathology EMB/obduction (age)	Outcome/follow-up (age)
C-VII-8	M	38C>T	Screening (42) Collapse (49)	Aspecific IV CD (42) Second-degree AVB LAHB/RBBB NSVT (49)	Normal (42) DCM, LV+RV dysfunction (R>L) LV hypertrabeculation (49)	Normal CK normal		ICD (49)
C-VII-9	M	OC					NA	Nocturnal SCD (40)
C-VII-10	M	38C>T	Distal myopathy (32)	VPBs cAVB, PM (40)	DCM (37)	Distal myopathy (Markesbery) (32) CK ↑↑	EMB: myocyte hypertrophy/fibrosis/ desmin aggregates EM: normal Muscle biopsy: myopathy, vacuoles	PM Home mechanical ventilation
C-VIII-1	M	38C>T	Screening (21)	RBBB Generalized repolarization disorder		Normal		
D-I-1	M	Likely			HF (37)			Died (37)
D-II-1	M	Likely	Chest pain (45)	Repolarization abnormality (45) Rhythm abnormality (48)	DCM (54)	NA CK normal		Died (55); progressive HF Possibly also CAD
D-II-2	M	OC				Unspecified myopathy (37) (dist>prox) CK normal	Muscle biopsy: myopathy	SCD (54)
D-II-3	M	OC	HF signs (53)	First-degree AVB (53)	DCM (53); LV+RV failure (53)	NA CK ↑↑	EMB: aspecific RV changes (53) Postmortem: fibrofatty replacement myocardium	Progressive HF (54), cardiomyoplasty (54), cAVB PM (54),[22] SCD 54
D-II-5	M	38C>T	Arrhythmias (30) Palpitations (47)	VPBs (30) LAHB/RBBB (47) AF (56) First-degree AVB (56)	DCM (56)	Muscular weakness (45) LGMD (52) Prox>distal legs CK ↑	Muscle biopsy (56): muscular dystrophy	ICD (62) CAD, PTCA (62) Appropriate ICD therapy (63) VTs
D-III-1	F	38C>T	Preoperation (27)	RBBB (27,34)	Normal (17,34)	NA		
D-III-2	F	38C>T	Collapse (41)	ECG abnormality (33) RBBB cAVB (41) PM	LVH (41)	NA		
E-I-2	F	38C>T	Muscular weakness (54)	Incomplete RBBB (62)	NA	Unspecified distal myopathy CK normal (54)		
E-II-1	M	38C>T	Muscular weakness (39)	cAVB, PM (33)		LG distribution weakness (36) CK ↑	Muscle biopsy: myopathic/dystrophic (39)	

Ages are given in years.

↑ <3 times normal value.

↑↑ <8 times normal value.

AF = atrial fibrillation; ARVC = arrhythmogenic right ventricular cardiomyopathy; AVB = atrioventricular block; CAD = coronary artery disease; cAVB = complete atrioventricular block; CM = cardiomyopathy; CK = creatine phosphokinase; DCM = dilated cardiomyopathy; EM = electron microscopy; EMB = endomyocardial biopsy; HF = heart failure; HTx = cardiac transplantation; ICD = implantable cardioverter-defibrillator; IVCD= intraventricular conduction delay; LAHB = left anterior hemiblock; LBBB = left bundle branch block; LG = limb–girdle; LGMD = limb–girdle muscular dystrophy; LV = left ventricular; LVH = left ventricular hypertrophy; NA = no information available; NSVT = nonsustained ventricular tachycardia; obj= objectivated; OC = obligate carrier; PM = pacemaker; PTCA = percutaneous transluminal coronary angioplasty; RV = right ventricle; RVOT = right ventricular outflow tract tachycardia; RBBB = right bundle branch block; SCD = sudden cardiac death; SVT = sustained ventricular tachycardia; TI= tricuspid insufficiency; VPB = ventricular premature beat; VT = ventricular tachycardia.

Table 2 ARVC task force criteria of two patients

Patient ID	Structural alterations	Tissue characterization	Repolarization abnormalities	Depolarization/conduction abnormalities	Arrhythmias	Family history
A VI-5	++	− (EMB; 21 years) ++ (after HTx; 27 years) Figure 2		++	+	+
A-VI-7	+	NA	+	+	+	++

The presence of two major, 1 major and 2 minor, or four minor criteria is sufficient to diagnose arrhythmogenic right ventricular cardiomyopathy (ARVC).
+ = minor criterion; ++ = major criterion.
EMB = endomyocardial biopsy; HTx = cardiac transplantation; NA = not available.

(range 14–53 years); nonsignificant]. The penetrance seems complete.

Spectrum of cardiomyopathies: Fifteen of 19 obligate and proven mutation carriers for whom information was available had developed a cardiomyopathic phenotype: 7 (47%) DCM; 5 (33%) left ventricular hypertrophy, diastolic dysfunction, or RCM-like phenotype; 1 (7%) unspecified form of cardiomyopathy; and 2 (13%) fulfilling ARVC task force criteria. In addition to these two latter patients (A-VI-5 and A-VI-7) who were diagnosed with ARVC, four other mutation carriers (A-VI-2, B-VI-9, C-VII-8, D-II-3) developed right ventricular (RV) heart failure during the course of the disease or in its early stages. RV involvement also was evident from the presence of right bundle branch block (RBBB) in 10 patients in whom it sometimes was the first or sole manifestation of early disease (A-VI-4, A-VII-4, B-IV-2, D-III-1, D-III-2, E-II-2) or from ventricular tachycardias originating in the RV (A-VII-3, A-VI-7).

Fifteen of 22 patients for whom data were available demonstrated RBBB, left bundle branch block, or AV block at the time of initial presentation, suggesting that conduction delay is an early manifestation of the disease.

The patients showed a severe clinical phenotype, including sudden cardiac death or progressive heart failure leading to early death or necessitating a heart transplant. Twelve of 27 mutation or obligate carriers died, underwent transplantation, or experienced appropriate implantable cardioverter-defibrillator interventions at a mean age of 48.9 years (range 27–63 years).

Neurologic: In 9 of 15 patients in whom neurologic investigations were performed, a neurologic disease was noted, mainly affecting the lower limbs. Muscular weakness was located proximally in two patients and more distally in five, suggesting a distal myopathy in some patients. A limb–girdle distribution was noted in two patients. CK values were normal or only mildly elevated (9 and 7 patients, respectively).

Genetic analysis

Sequence analysis of the *DES* gene revealed the missense mutation c.38C>T, leading to a serine to phenylalanine substitution at codon 13 (p.S13F) in the head domain of the desmin protein in all the index patients and relatives indicated in Table 1. This mutation alters a highly conserved residue, changes polarity, cosegregates with the disease, and was absent in 300 ethnically matched control alleles. Mutations in major genes underlying ARVC (*PKP2*, *DSG2*, *DSC2, DSP*) in patient A-VI-7 were excluded (data not shown). Seven unaffected relatives were genetically studied, but the c.38C>T mutation was not found.

Genealogy

Genealogic investigations revealed a common ancestral couple living around AD 1800 for families A, B, and C (Figure 1). Ancestors from families D and E could be traced back to the same small, poorly populated region in which the ancestral couple lived.

Haplotype analyses

Haplotype analysis around the *DES* gene demonstrated an identical haplotype for eight markers located in a 2.7-Mb region (Table 3). This haplotype was not, or was only partially, seen in controls and an unaffected family member. Based on haplotype and considering 25 years per generation, the age of the mutation is estimated to be between 220 and 495 years old.[19]

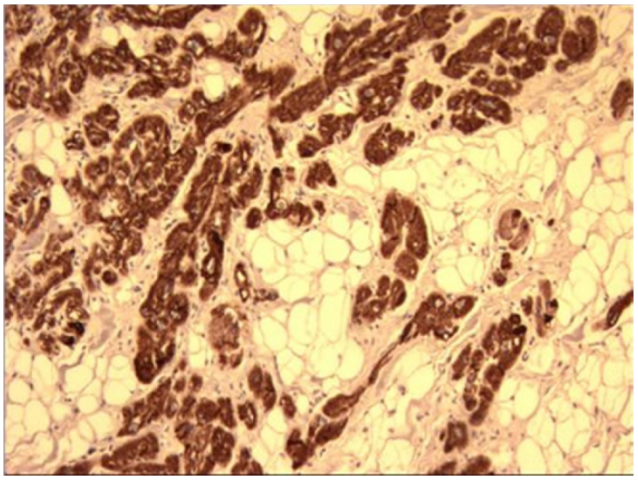

Figure 2 Histology of a right ventricular sample from patient A-VI-5 after cardiac transplantation showing signs of lipofibromatosis compatible with a diagnosis of arrhythmogenic right ventricular cardiomyopathy.

Table 3 Haplotype analyses of seven patients and one unaffected family member

Marker	A-VI-2	B-VI-9	B-VI-10	C-VII-8	D-II-5	Unaffected family D	E-I-2	E-II-1
D2S1384	152/156	138/152	143/151	147/152	147/138	143/156	147/156	152/156
D2S1380	120/140	140/144	128/140	120/120	120/120	120/144	120/120	120/120
D2S2382	261/261	255/261	255/261	251/245	251/255	245/245	251/255	251/245
D2S434	266/266	266/270	266/266	266/266	266/266	266/270	266/274	266/270
D2S104	115/115	115/111	115/111	115/111	115/111	111/117	115/117	115/115
D2S1338	172/192	172/184	172/164	172/164	172/168	164/196	172/192	172/172
D2S2244	240/236	240/238	240/238	240/238	240/236	236/236	240/234	240/238
DES-gene	38C>T	38C>T	38C>T	38C>T	38C>T	None	38C>T	38C>T
D2S2151	250/250	250/248	250/248	250/250	250/250	250/248	250/250	250/250
D2S163	223/217	223/219	223/219	223/217	223/225	223/223	223/217	223/215
D2S2359	270/265	270/267	270/267	270/265	270/265	265/267	270/265	270/266
D2S1242	157/148	157/161	157/161	157/152	157/165	164/168	157/156	157/161
D2S130	192/192	192/200	192/200	192/196	198/198	192/198	192/198	192/192
D2S1363	169/185	177/177	177/177	169/169	169/185	177/177	177/189	177/177
D2S2354	255/267	253/253	253/253	253/253	253/269	253/257	253/253	253/263

Haplotypes shared by mutation carriers are shown in *gray shaded areas*. Affected persons show a common haplotype spanning a 2.7-Mb region around the *DES* gene.

Histology, immunohistochemistry, and electron microscopy

Heart tissue from the left ventricle and RV of patients A-VI-5, B-VI-9, D-II-3, and C-VII-10 had large aggregates of desmin filaments in the sarcoplasm of some cardiomyocytes (Figure 3A). To a lesser extent, either large or granular accumulation of protein sequestosome-1 was seen in several cardiomyocytes (Figure 3B), confirming earlier observations that sequestosomes are involved in desminopathies.[23] Desmin deposits in skeletal muscle in B-VI-9 and B-VI-10 were described previously.[12]

Immunohistochemistry in patients A-VI-2, B-VI-9, and D-II-3 showed that the amounts of immunoreactive signal for the major adherens junction protein N-cadherin and the major desmosomal proteins plakophilin-2, desmoplakin, plakoglobin, and desmocollin-2 were comparable to those in controls (Figure 4). However, the major gap junction protein connexin43 (Cx43) was reduced in B-VI-9 (Figure 4), a finding not observed in the other patients. Ventricular myocytes from affected persons showed modest derangements in desmin distribution but an abnormal structure of intercalated disks (Figure 4). In control myocardium, junctional proteins appeared in straight robust lines of high intensity, whereas in myocardium from mutation carriers, the shape of the intercalated disk was disturbed, appearing convoluted and elongated with a strong zigzag pattern (Figure 4). These findings were absent in two normal controls and in other patients with idiopathic DCM.

These highly irregular and twisted intercalated disks were also seen by electron microscopy in samples from D-II-3 and C-VII-10 (Figure 5). We cannot exclude that modest structural changes in the insertion of sarcomeres at the intercalated disk are present, but this finding also could be artifactual. Notably, the Z-disks appeared to be aligned.

Discussion

Disease-associated *DES* mutations are generally located in the α-helical domain of desmin, and only five mutations in the N-terminal head domain have been described to date.[2,10,24,25] The head domain is believed to be important for aggregation of tetramers to fibers (assembly) and for stability of the protein. Interaction with the outer cellular membrane occurs through the desmosome where desmin interacts with several proteins such as desmoplakin.[1,26] The assembly state of desmin seems to affect binding to desmoplakin, suggesting that desmin has a role in interaction with the desmosome.[27]

We recently described in detail the index patients from families B and D.[12] This identical mutation has also been identified in four patients from a two-generation Chinese family.[28] The present study covers the largest series of patients with a single *DES* mutation. It summarizes the details of 27 proven or obligate carriers of this founder mutation and four additional family members who probably were affected and highlights additional immunohistochemical myocardial features.

Cardiac phenotype

All patients carrying the c.38C>T mutation for whom sufficient data were available had a cardiac phenotype. In 19 (86%) of 22 patients, cardiac symptoms were the initial manifestation. This number differs significantly from recently reported data of cardiac involvement found in 67 of 92 patients ($P < .05$).[11] Therefore, we conclude that the c.38C>T specific mutation is predominantly associated with cardiac pathology. Nearly 80% (15/19) of patients developed a cardiomyopathic phenotype, with DCM being most prevalent (7/15 [47%]). The predominance of DCM agrees with a recent review that found DCM in 52 (57%) of 92 cases verified at the molecular level.[11]

Our observation of patients fulfilling ARVC criteria has not been previously reported. Although right sided heart failure in *DES* mutation carriers has occasionally been reported in the literature, it was present in four c.38C>T mutation carriers in addition to the 2 carriers fulfilling the ARVC criteria.[29–31]

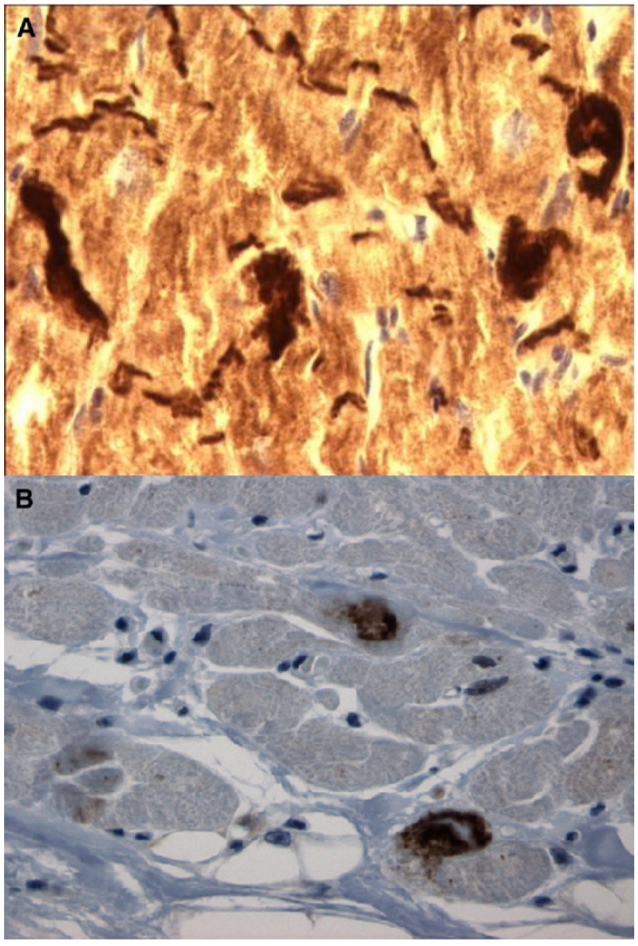

Figure 3 Myocardial tissue from patients A-VI-5 (transplantation), B-VI-9 (postmortem), C-VII-10 (endomyocardial biopsy), and D-II-3 (postmortem) showing desmin aggregates in the sarcoplasm of some cardiomyocytes **(A)** and large or granular accumulation of protein sequestosome-1 **(B).**

Because desmin is highly expressed in conductive myocytes, it is easy to accept that conduction disorders, including RBBB, could be the initial manifestation of a desminopathy.[32,33] This was also seen in our population, as left bundle branch block, RBBB, or atrioventricular block was observed in 15 of 22 patients at the time of initial diagnosis.

Neurologic disease

The proportion of patients in our cohort who also demonstrated signs of a myopathy (9/15) seems lower than the combined data from a study of 92 desmin mutation carriers, of whom 80% demonstrated a myopathy.[11] Moreover, we confirmed that CK measurement does not necessarily help in diagnosing a desminopathy, even in patients with generalized neurologic disease.[3,10]

Because some patients were diagnosed with other neurologic diseases, such as distal myopathy or limb girdle muscular dystrophy, we suggest that *DES* screening be considered for patients with these diagnoses in association with cardiologic symptoms.

Histology–immunohistochemistry

The novel observation of frequent RV involvement in *DES* *c.*38C>T mutation carriers (and given that desmosome cardiomyopathies are predominantly right-sided) suggests that the effect of the c.38C>T mutation might be at the level of desmin–desmosome interaction. Moreover, *DES* null mice demonstrating extensive RV involvement that progresses after exercise and mice expressing a 7-amino-acid deletion of desmin also show reduced desmosomal and adherens junction proteins, suggesting a role for desmin in RV heart failure at the level of the intercalated disk.[34–37] In Carvajal syndrome, a recessive cardiocutaneous syndrome caused by a homozygous desmoplakin deletion, highly convoluted intercalated disks and reduced levels of desmoplakin, plakoglobin, and desmin signals have been found.[38] This suggests a common pathway in both desminopathies due to the c.38C>T mutation and desmosome cardiomyopathies, possibly attributed to an interaction of desmin and desmosomal proteins (desmoplakin in particular) with a possible reduction of desmosomal proteins at the intercalated disk. We hypothesized that this reduction might also underlie the c.38C>T phenotype, but the levels of immunoreactive signal of the desmosomal proteins tested were normal in all of our patients. However, the distribution pattern of desmosomal proteins was disrupted, leading to intercalated disks that were more convoluted and elongated with a zigzag appearance. This finding was confirmed by electron microscopic studies of samples from C-VII-10 and D-II-3, which possibly also demonstrated some structural changes at the insertion of sarcomeres at the intercalated disks. The myofibrils seemed aligned, so the effect of the mutation in the head domain of *DES* might be due to a more localized misalignment of cytoskeletal elements. A mouse model of a desmin cardiomyopathy showed that when formation of desmin filaments is grossly disrupted, the sarcomeres become misaligned in such a way that the Z-disks become

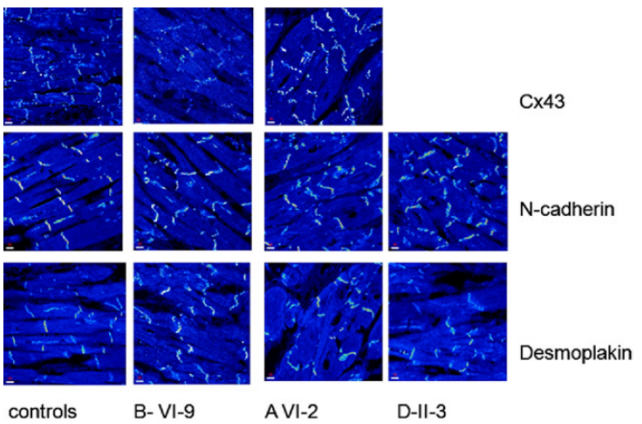

Figure 4 Immunofluorescence samples from patients B-VI-9, A-VI-2, and D-II-3 suggesting reduced amounts of connexin 43 (Cx43) in patient B-VI-9, who had end-stage right ventricular failure; equal amounts of immunoreactive signal compared with controls for the major adherens junction proteins N-cadherin and desmoplakin, and intercalated disks with an abnormal structure. The intercalated disks had a zigzag pattern and appeared more convoluted and elongated than normal.

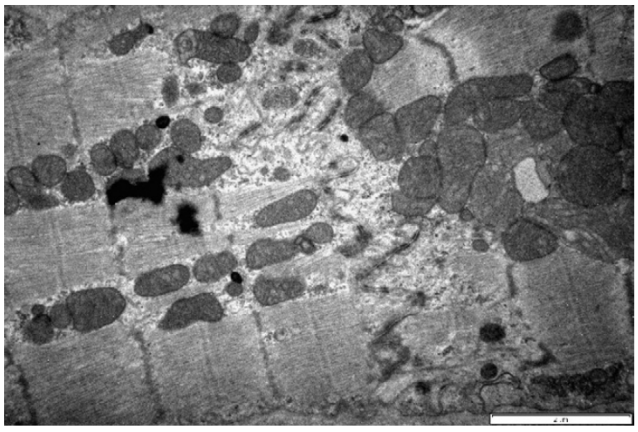

Figure 5 Electron microscopy of myocardial tissue from patient C-VII-10 showing irregular and bent intercalated disks and possibly some structural changes in the insertion at the disks. The Z-disks appear to be aligned, suggesting that the sarcomeres are in register.

staggered.[39] Desmin null mice also showed zigzag intercalated disks.[34] The changes observed in our patients were not identified in patients with dilated, hypertrophic, or ischemic end-stage cardiac disease or in normal controls.[40]

A possible reduction of Cx43 was seen in patient B-VI-9. A defect in cell–cell adhesion or linkage between intercellular junctions and cytoskeleton could prevent normal localization of connexins, not the expression of Cx43 in itself, in gap junctions underlying tachyarrhythmias. This was also found in some cardiocutaneous syndromes and a mouse model of desmin-related myopathy.[37,38,4,41,42] However, other than the shape of the intercalated disks, the localization and distribution of desmosomal proteins was not affected in patient B-VI-9; therefore, Cx43 down-regulation has to be considered a result of the patient's late-stage heart failure. This may underlie ventricular arrhythmias and hence sudden death, which is generally not an initial feature of disease in *DES* c.38C>T mutation carriers.

Pathophysiology of the c.38C>T mutation

The Ser13 residue is a phosphorylation site for protein kinase-C[43] and is believed to be important in the regulation of desmin filament assembly and disassembly.[43,44] Results of experiments with cultured hamster cells indicate that disassembly of desmin could be caused by protein kinase-C–mediated phosphorylation leading to myofibril disarray in cardiomyopathic cells, as the role of desmin in maintaining myofibril alignment was disturbed.[44] However, transfection studies showed that, contrary to other *DES* mutations, the p.S13F mutant preserved its filamentous network.[28] These results and our observations indicate that no major misalignment of myofibrils occurs. The observed changes in intercalated disks might be caused by instability or a mild/partial defect of the desmin filamentous network with desmin accumulation.[28] Preliminary data on the functional aspects of head domain mutations of desmin have shown that, during *in vitro* assembly (also combined with wild-type desmin), unit-length filament formation and elongation are conserved, although abnormal structures are found. Notably, in transfected cells some mutations distort the regular arrangement of exogenous and endogenous proteins.[45]

From the pathophysiologic point of view, our observation of abnormalities in the intercalated disk region is important. It suggests that interactions between desmin filaments and components involved in intercalated disk organization are impaired. We do not yet know whether interactions between the head domain and other components are affected or if, through effects of the p.S13F mutation on the structure of desmin, interactions of other domains of the desmin molecule with components such as desmoplakin are affected.

Conclusion

This report describes the largest series to date of 27 patients with a single head domain desmin mutation. The associated phenotype is fully penetrant. Patients demonstrated a highly variable yet predominantly cardiologic clinical picture of right-sided myocardial involvement (including the diagnosis of ARVC), RBBB, and/or ventricular tachycardias originating from the RV during the early phases of the disease. Males manifested disease at an earlier age than did females. Some of the families showed little neurologic disease. Immunohistochemistry demonstrated an effect of the mutated protein on the regions where junctional proteins reside. The study found more convoluted and elongated intercalated disks, suggesting a localized effect of the mutant protein on cellular connections that may, apart from effects of desmin aggregates, also contribute to the pathogenesis of desmin head domain mutations. Given the clinical overlap with desmosome cardiomyopathies as demonstrated in these families, we postulate a common pathway for these two entities.

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