Inherited cardiomyopathies

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Chapter 7

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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In preparation

Abstract

Background: Desmin-related myopathy is a clinically heterogeneous group of disorders encompassing myopathies, cardiomyopathies and/or conduction disease and combinations of all of these. Mutations in the gene encoding desmin (*DES*), a major intermediate filament, can underlie this phenotype. We recently identified five families with an identical mutation in the head domain region (p.S13F) of desmin and studied their clinical and pathological characteristics.

Methods and results: We studied 27 patients, who are all carriers or obligate carriers of a p.S13F *DES* founder mutation; they demonstrated a variable phenotype, but all had cardiac disorders. The clinical picture was characterized by high-grade AV (atrio-ventricular) blocks at a young age and important right ventricular (RV) involvement. This RV predominance is demonstrated by the presence of a right bundle branch block (RBBB) in 10 patients (sometimes as a first manifestation), and RV heart failure in six patients, including two who fulfilled the diagnostic criteria for arrhythmogenic right ventricular cardiomyopathy (ARVC). Because of this clinical overlap with desmosome cardiomyopathies, we also studied desmosomal proteins and the intercalated disks. Normal amounts of the major desmosomal proteins were found, yet the intercalated disks were more convoluted and elongated with a zigzag appearance.

Conclusions: This is the largest series of patients described with a single head domain *DES* mutation. They show a variable, yet predominantly cardiological phenotype, characterized by conduction-disease at early age and RV involvement including RBBB and/or ventricular tachycardias originating from the RV and ARVC-phenocopies. A localized effect of desmin on the intercalated disks might contribute to the pathogenesis.

INTRODUCTION

The *DES* gene encodes desmin, a major intermediate filament of skeletal and cardiac muscle, which provides structural and functional integrity by transmitting mechanical stress, organelle positioning, organization and assembly of sarcomeres, and signal transduction and apoptosis.[1] Mutations in the desmin (*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 and restrictive cardiomyopathies, DCM and RCM respectively), cardiac conduction disease (CCD) and combinations

thereof.[2-10] If both cardiological and neurological features occur, these can manifest in any order as cardiological features can precede, occur simultaneously with, or after the manifestation of generalized neuromuscular disease.

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

We recently identified five Dutch families (with 27 affected individuals) with a variable, yet predominant cardiological phenotype seen in patients carrying an identical missense mutation in the head domain of the *DES* gene. Two of these families have been partially described.[12] We here report on: (1) the wide phenotypic variability in the largest series of patients with a single *DES* mutation, which 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 typical myocardial immunohistochemistry findings.

SUBJECTS AND METHODS

Clinical evaluation

The five index patients were referred to our cardiogenetics outpatient clinic, UMC Groningen (UMCG), with either a primary cardiological phenotype (2 index patients) or a neurological phenotype associated with cardiac manifestations in three index patients. Two of the index patients were recently described by Bergman et al.[12] The index patients and their relatives underwent a regular clinical genetic counseling procedure. Those who gave informed consent were evaluated for cardiological and/or neurological and genetic characteristics.

All probands and family members were evaluated cardiologically by a 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] To diagnose DCM, we used the criteria and methods of investigation proposed by Mestroni et al.[14] and for ARVC, we used the generally accepted task force criteria.[15]

In patients with a primary cardiological phenotype, the initial neuromuscular examination was restricted to taking a patient and family case history of neuromuscular complaints, a physical examination by an experienced neurologist and creatine phosphokinase (CK) measurement. If these were negative, no additional neurological 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's Medical Ethics Committee.

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

Genealogy

To discover if there were any distant relationships between the index patients, their genealogy was investigated using data from civil registers and state archives; their pedigrees were reconstructed to around AD 1800 (covering 6-8 generations).

Haplotype analysis

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

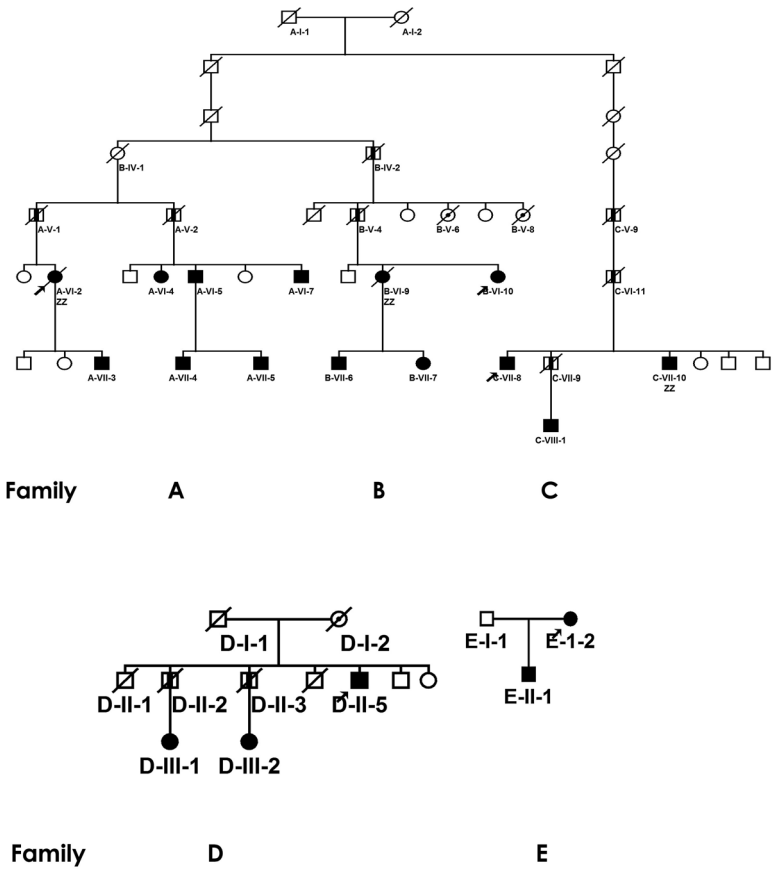

Figure 1. Pedigrees of the families studied. Square symbols indicate men; circles women. Black symbols: clinical signs and 38C>T mutation; vertical bar in symbol means obligate carrier of the 38C>T mutation; diagonal line through symbol indicates deceased; point in symbol: signs of desminopathy but not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

Histology, immunohistochemistry and immunofluorescence

In three patients (A-VI-2, B-VI-9 and 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 (Dept. of Pathology, Groningen, the Netherlands) 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 Benchmark immunostainer was used according to the manufacturer's protocols (Ventana Medical Systems Inc., Tucson, AZ, USA). Appropriate positive and negative controls were used.

Immunostaining of patient samples and two controls was performed (Department of Pathology, Boston, 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 Cx-43 (SIGMA, St. Louis, MO, USA) rabbit polyclonal anti-desmoplakin (SEROTEC, Kidlington, UK) and rabbit polyclonal anti-desmin (AbCam, Cambridge, UK), and secondary antibodies cyanine 3 (Cy3) conjugate affini-pure goat anti-mouse/anti-rabbit IgG's (Jackson Immunoresearch, West Grove, PA, USA). Immunostained preparations were analyzed by laser-scanning confocal microscopy (Sarasto model 2000, molecular dynamics) as previously described.[20]

For electron microscopy (UMCG, the Netherlands), endomyocardial biopsies were processed by fixation in 2% buffered glutaraldehyde and stained with 1% osmium tetroxide, after which material was embedded in Epon 812 (Serva, Heidelberg, Germany).

RESULTS

Clinical results

Phenotypic description

Detailed clinical data of all individual patients, including outcomes and results of previous histology, are presented in Tables 1 and 2 and the pedigrees are shown in Figure 1. Results of clinical evaluation were available for 22 individuals from the five families, but only limited information was available for five more patients, who were obligate mutation carriers.

Family A: Patient A-VI-2 came to our attention because of an intermittent total atrio-ventricular block (AVB), for which a pacemaker was implanted. She also developed atrial fibrillation (AF) and atrial flutter, and eventually she developed right and left ventricular (RV and LV) failure and died due to end-stage heart failure. Her son (A-VII-3) (Fig. 1) was evaluated because of exercise-related palpitations accompanied by dizziness. During exercise testing he developed a left bundle branch block (LBBB) ventricular tachycardia (VT), suggesting an RV origin.[21] His echocardiogram has remained normal during 8 years of follow up.

The further family history was remarkable: a paternal cousin (A-VI-5) of the index patient underwent a cardiac transplantation at age 27 years because of end-stage RV and LV failure. At presentation at age 21 he fulfilled the criteria for ARVC (Table 3). The explanted heart demonstrated dilatation of the RV outflow tract with fibrofatty replacement of myocardium and thinning of the ventricular wall and LV apex abnormalities, compatible with ARVC (Figure 2) (Table 3).

His 38 year-old brother (A-VI-7, Fig. 1) was recently admitted because of a collapse. Examinations revealed a low voltage ECG with negative T-waves in V2 and V3, and an impaired RV and LV function with an enlarged RV diameter. During exercise testing he developed a LBBB VT. He also fulfilled the criteria for ARVC (Table 3).[21] A sister, A-VI-4, was evaluated at age 50 years. She had a borderline low voltage ECG with an RBBB (Figure 3). During exercise testing, she developed premature ventricular beats (PVBs) with a LBBB morphology, which were also seen during Holter monitoring. The son (A-VII-4) of the transplanted patient was evaluated at age 11 years because of the family history. Three years later his ECG showed repolarization

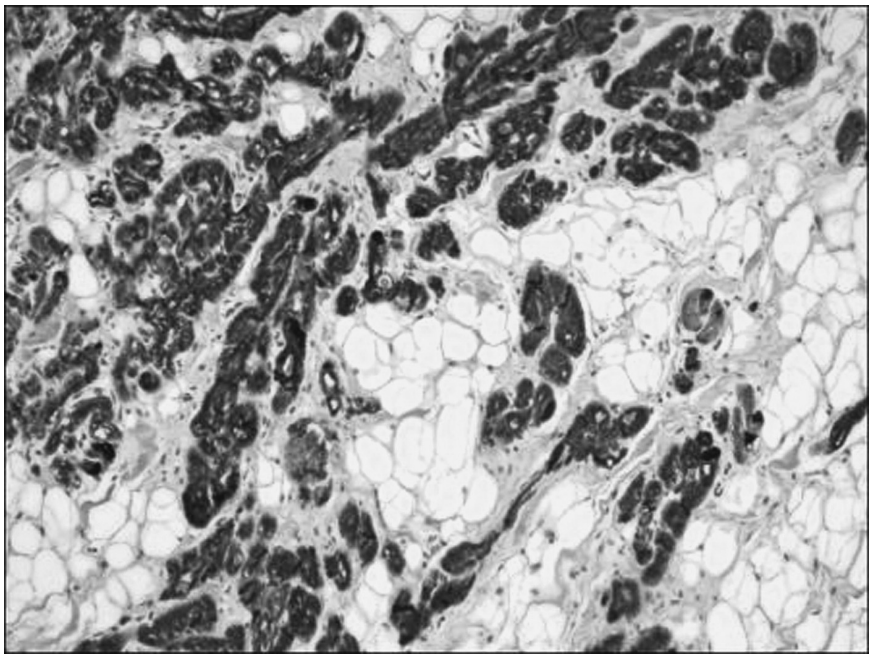

Figure 2. Histology of a right ventricular sample (patient A-VI-5) after cardiac transplantation showing signs of lipofibromatosis compatible with a diagnosis of ARVC. (color image: page 268)

Table 1: clinical features of proven or obligate DES 38C>T carriers

Patient ID	gender	Genetic status	Referred for (age)	Cardiological ECG/Rhythm (age)	Cardiological 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	f	38C>T	palpitations	cAVB (40) PM AF(47) NSVT	DCM (54) RV>LV	nl	EMB: aspecific abn/ Hypertrophy	Died (58) progr RV failure
A-VI-4	f	38C>T	Screening (50)	L/RBBB(50) low-voltage(52) VPBs (52)	left atrium size↑	mild prox. myopathy legs(52) CK:nl		Hypertension Palpitations (52)
A-VI-5	m	38C>T	Heart failure (21)	Low voltage, RBBB, AF (21)	HF RV>LV(21) TI(22)	mild pnp (43) CK nl	EMB (21) fibrosis Explanted heart : dilated RVOT; ARVC (Fig 2)	HtX(27) Lymphoma (38); chemotherapy
A-VI-7	m	38C>T	Collapse (37)	LBBB VTs(37)	ARVC TFC+ DCM (37)	nl (37); CK nl		ICD (37) 2 yrs uneventful
A-VII-3	m	38C>T	Palpitations (<17)	Repolarization abn. VPBs, VTs (18)	nl (28)	nl (28); CK nl		Stable (26); ICD (28);
A-VII-4	m	38C>T	screening (14)	Repolarization abn. RBBB VPBs (18)	RCM-like (15) LVH	na; CK nl		Palpitations impaired exercise tolerance
A-VII-5	m	38C>T	screening (11): nl	Repolarization abn Epsilon wave, VT's(14)	nl (14); mild diast dysfunction	nl; CK nl (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 pnp CK na		CAD; died HF (60)
B-VI-9	f	38C>T	Palpitations (±27)	cAVB/PM (±27) VT (30) AF (49)	Unspec. CM (±27) RV HF (51)	Dist>Prox (36) LGMD CK ↑	EMB (51): myocyte hypertrophy/ fibrosis; desmin aggr. EMB RV dilatation, fibrosis, desmin aggr	Died RV HF (51)
B-VI-10	f	38C>T	Palpitations VPBs (42)	VT;SVT; VPBs LAHB (43) cAVB/PM (45)	nl (45/47)	prox>dist musc. Weakness (30) (obj 45 yrs) LG distribution CK ↑-↑↑	MB: desmine accumulation	Alive 50
B-VII-6	m	38C>T	collapse (31)	cAVB PM (31)	Mild LVH, diast LV dysfunction (33)	na		No progression (35)

B-VII-7	f	38C>T	collapse (35)	cAVB PM (37)	Mild diast LV dysfunction (37)	na CK: ↑		
C-VI-11	m	OC						Died in Unexplained accident (46)
C-VII-8	m	38C>T	Collapse (49)	Aspec IV CD (42) 2nd AVB LAHB/RBBB NSVT (49)	nl (42) DCM, LV+RV dysf (R>L) Hypertrabec.(49)	nl ; CK nl		ICD (49)
C-VII-9	m	OC					na	Noctural SCD 40
C-VII-10	m	38C>T	Distal myopathy (32)	VPBs cAVB, PM (40)	DCM(37)	Distal myopathy (markesberry) (32) CK ↑↑	EMB:myocyte hypertr/fibrosis/DES aggregates EM: nl Musc biopsy: myopathy; vacuoles	PM Home mechanical ventilation
C-VIII-1	m	38C>T	Screening (21)	RBBB Gen. repolarisation disorder		nl		
D-II-2	m	OC				Unspec. Myopathy (37) (dist>prox) CK: nl	MB: myopathy	SCD (54)
D-II-3	m	OC	HF signs (53)	1st AVB	DCM (53); LV+RV failure (53)	na; CK↑↑	EMB:aspecific changes RV (53) Postm: fibrofatty replacement myocardium	Prog 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) 1st AVB (56)	DCM (56)	Musc. weakness (45) LGMD (52) CK ↑	MB (56): muscular dystrophy	ICD (62) CAD, PTCA (62) Appropriate ICD therapy (63) VTs
D-III-1	f	38C>T	27 pre-operation	RBBB (27, 34)	nl (17,34)	na		
D-III-2	f	38C>T	Collapse (41)	ECG abn (33) RBBB cAVB (41) PM	LVH (41)	na		
E-I-2	f	38C>T	Musc. weakness (54)	Incompl RBBB (62)	na	Unspec. distal myopathy CK=nl		
E-II-1	m	38C>T	Musc weakness(39)	cAVB, PM (33)		LG distribution weakness(36) CK ↑	MB: myopathic/dystrophic (39)	

Abn, abnormal; Aggr, aggregates; AVB, atrioventricular block (1st, 2nd, complete); CAD, coronary artery disease; CM, cardiomyopathy; CK, creatinine phosphokinase; Diast, diastolic; DCM, dilated cardiomyopathy; EMB, endomyocardial biopsy; Gen, generalized ;HF, heart failure; HTx, cardiac transplantation; ICD, implantable cardioverter defibrillator; LAHB, left anterior hemiblock; LG, limb-girdle; LGMD, limb girdle muscular dystrophy; LV, left ventricular; LVH, left ventricular hypertrophy; MB, muscular biopsy; musc, muscular; na, no information available; nl, normal; NSVT, nonsustained ventricular tachycardia; OC, obligate carrier; PM, pacemaker; PME, post mortem examination; PNP, polyneuropathy; Prog, progressive; PTCA, percutaneous transluminal coronary angioplasty; RV, right ventricle; RVOT, right ventricular outflow-tract tachycardia; RBBB, right bundle branch block; SCD, sudden cardiac death; unspec, unspecified; VPB, ventricular premature beat; VT, ventricular tachycardia; ↑, <3 times normal value; ↑↑, <8 times normal value

Table 2: clinical features of family members likely to be carrier of the DES 38C>T mutation.

Patient ID	gender	Genetic status	Referred for (age)	Cardiological ECG/Rhythm (age)	Cardiological Structural (age)	Neuromuscular phenotype (age)	Pathology EMB/obduction (age)	Outcome/follow up (age)
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: mild aspecific abn., fibrosis (46) musc. dystrohpy (52)	CAD (59) died myocardial infarction (59)
D-I-1	m	likely			HF (37)			died (37)
D-II-1	m	likely	Chest pain (45)	Repolarziation abn (45) Rhythm abn (48)	DCM (54)	na; CK=nl		died (55); progr HF Possibly also CAD

Abbreviations as in Table 1.

Table 3: ARVC task force criteria of two patients; ++, major criterion; +, minor criterion.

	Structural alterations	Tissue characterization	Repolarization abnormalities	Depolarization/conduction abnormalities	Arrhythmias	Family history
A-VI-5	++	- (EMB; 21yrs) ++(after HtX;27 jrs)		++	+	+
A-VI-7	+	na	+	+	+	++

The presence of either 2 major, 1 major and 2 minor or 4 minor criteria is sufficient to diagnose ARVC. Na, not available; EMB, endomyocardial biopsy; Htx, cardiac transplantation.

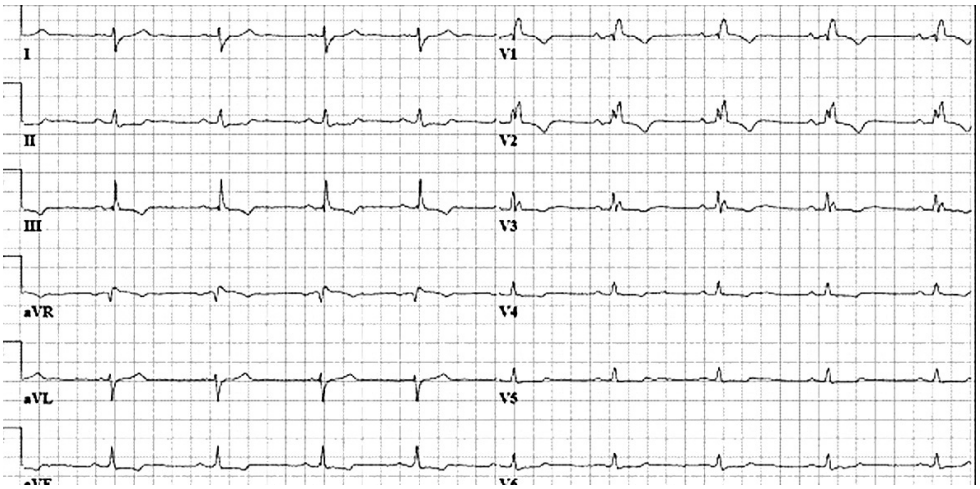

Figure 3. ECG of patient A-VI-4 showing low voltage and an RBBB.

abnormalities, echocardiography revealed mild LV hypertrophy with diastolic dysfunction, and Holter monitoring showed monomorphic PVBs with an RBBB configuration. The ECG of his brother (A-VII-5, Figure 1), age 14 years, showed an epsilon wave and abnormal repolarization. Echocardiography revealed mild diastolic dysfunction. He had an impaired exercise tolerance but no exercise-related ventricular arrhythmias. During follow up, Holter monitoring showed an RBBB monomorphic VT. He received an ICD at age 15 years because of a serious collapse, probably due to a VT.

Family B*:* The female index patient in family B (B-VI-10) has recently been described.[12] In short, she presented with muscular weakness suggesting limb-girdle muscular dystrophy (LGMD), which she had had since age 30 years. At 43 years, palpitations due to supraventricular tachycardias and PVBs were noticed. Two years later she collapsed and a left-anterior hemiblock (LAHB) progressing to a total AVB was apparent (Table 1). Her sister (B-VI-9) demonstrated electrocardiographic signs of an unspecified cardiomyopathy and had had tachycardias from the age of 27 years combined with a complete AVB. She developed progressive RV failure (49 years), which was the cause of her death at age 51 years. Postmortem examination revealed a dilated RV with hypertrophic cardiomyocytes as well as degenerated cardiomyocytes with central vacuolar changes and dense aggregates of desmin.[12] Similar clinical entities had been present in their father (B-V-4) (Table 1) and two aunts (B-V-6 and B-V-8, Table 2). Her son (B-VII-6) presented at age 31 years with a collapse due to a total AVB; echocardiography revealed

mild LV hypertrophy and slightly disturbed diastolic dysfunction. Recently her 37 year-old daughter (B-VII-7) was admitted because of a serious collapse with a total AVB with pauses up to 18 seconds. Echocardiography revealed mild diastolic dysfunction but no other signs of cardiomyopathy.

Family C: The 49 year-old index patient (C-VII-8) collapsed while in rest. Investigations revealed a non-sustained VT (NSVT) and a second-degree AVB. Echocardiography and LV angiography demonstrated signs of dilated cardiomyopathy and LV hypertrabecularization. Seven years before cardiac evaluation, because of nocturnal SCD in his 40-year-old brother (C-VII-9), he only demonstrated aspecific intraventricular conduction delay. Another brother (C-VII-10) (Fig. 1) had signs of a distal myopathy, DCM and a total AVB. Their father (C-VI-11) (Fig. 1) had died in an unexplained car accident, aged 46 years. An ECG in his 22 year-old nephew, C-VIII-1, revealed an RBBB and a generalized repolarization disorder. Neurological investigations were normal.

Family D: The index patient (D-II-5) was referred for DCM, AF, first-degree AVB and complete RBBB at age 56 years. Arrhythmias had, however, been present since age 30 years. Progressive weakness and atrophy of the proximal (more than distal) leg muscles was found at age 52 years.[12] His brother (D-II-3) presented at age 53 years with DCM and first-degree AVB. Subsequently he developed heart failure and underwent a cardiomyoplasty. Three months after the operation he developed a total AVB requiring pacemaker therapy.[22] He died suddenly aged 54 years. A similar picture was seen in another brother (D-II-1) 45 years of age, with aspecific ECG abnormalities, followed by heart failure 8 years later (Table 2). He died due to progressive heart failure aged 55 years. The details of two other proven mutation carriers (D-III-1 and D-III-2) from this family are summarized in Table 1, with an RBBB in D-III-1 as the sole manifestation at age 34 years.

Family E: The index patient (E-I-2, Fig. 1) was referred for proximal muscular weakness due to an unspecified form of (distal) myopathy at age 54 years. An ECG at age 62 years demonstrated an incomplete RBBB. Her son (E-II-1) had received a pacemaker at age 33 years for an AVB. From the age of 36 he noted muscular weakness and at age 39, a limb-girdle-like distribution of muscular weakness was found. Muscular biopsy was compatible with a desminopathy.

Genetic analysis

Sequence analysis of the *DES* gene revealed a 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. The mutation identified 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* and *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

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

Haplotype analyses

Haplotype analysis around the *DES* gene demonstrated an identical haplotype for 8 markers located in a 2.7 Mb region (Table 4). This haplotype was not, or only partially, seen in controls and an unaffected family member. Based upon haplotype and some 25 years per generation, the age of the mutation is estimated to be between 220 and 495 years old.[19]

Table 4. Haplotype analyses of 7 patients and one unaffected family member.

Marker	A-VI-2	B-VI-9	B-VI-10	C-VII-8	D-II-5	Unaffected Fam 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 in gray shaded areas. Affected persons show a (partly) common haplotype spanning a 2.7Mb region around the *DES* gene

Histology, immunohistochemistry and electron microscopy

Heart tissue from the LV and RV of patients A-VI-5, B-VI-9, D-II-3 and C-VII-10 revealed large aggregates of desmin filaments in the sarcoplasm of some cardiomyocytes (Fig. 4A). To a lesser extent, either large or granular accumulations of protein sequestosome-1 were seen in several cardiomyocytes (Fig. 4B), which confirmed earlier observations that sequestosomes are involved in desminopathies. Desmin deposits in skeletal muscle in B-VI-9 and B-VI-10 were described earlier.[12]

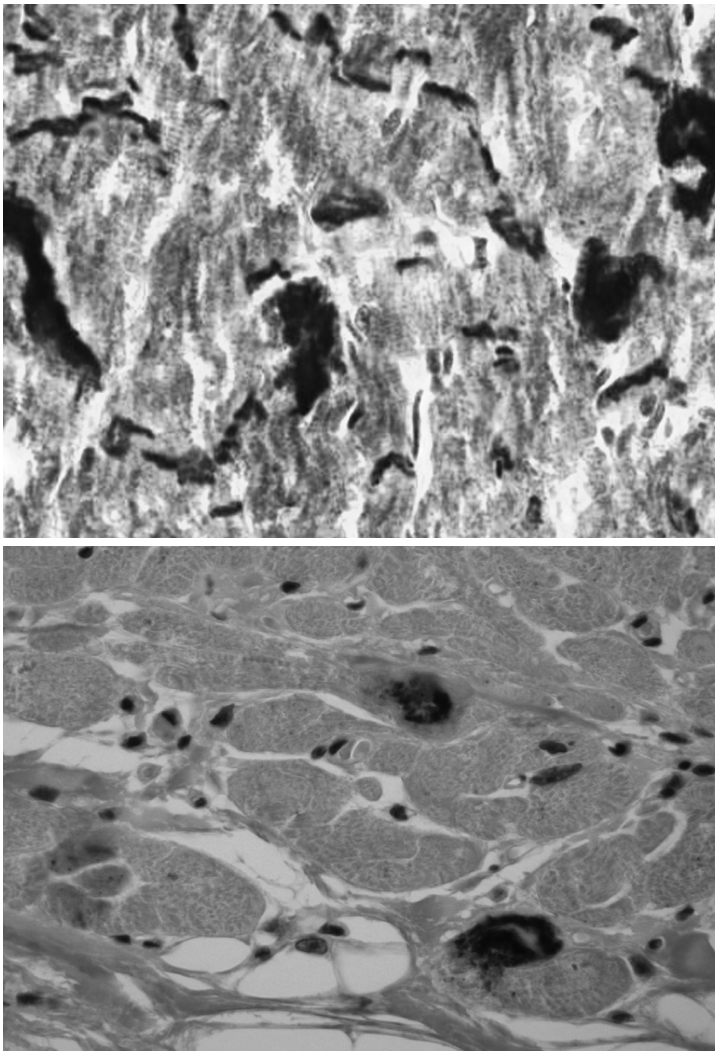

Figure 4. Myocardial tissue from patients A-VI-5 (transplantation), B-VI-9 (postmortem), C-VII-10 (endomyocardial biopsy), and D-II-3 (postmortem) show desmin aggregates in the sarcoplasm of some cardiomyocytes (panel A) and large or granular accumulations of protein sequestosome-1 (panel B). (color image: page 269)

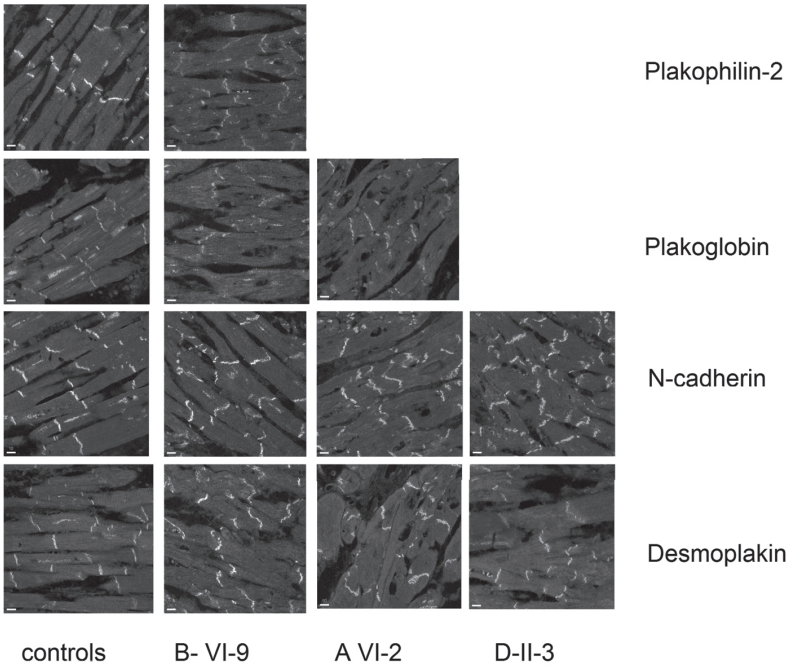

Figure 5. Myocardial samples from patients B-VI-9, A-VI-2 and D-II-3 demonstrating equal amounts of immunoreactive signal compared to controls for the major adherens junction proteins palkophilin-2, plakoglobin, N-cadherin, and desmoplakin, with an abnormal structure of intercalated disks that showed a zigzag pattern and appeared more convoluted and elongated than normal. (color image: page 270)

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 major desmosomal proteins (plakophilin-2, desmoplakin, plakoglobin, desmocollin-2) were comparable to those in controls (Fig. 5). The major gap-junction protein Cx43 was, however, reduced in B-VI-9 (Fig. 6), whereas this was not noticed in the other patients. The ventricular myocytes from affected persons exhibited modest derangement in desmin distribution, but with an abnormal structure of intercalated disks (Fig. 5). In control myocardium, junctional proteins showed straight robust lines of high intensity whereas in the mutation carriers, the shape of the intercalated disk was disturbed, appearing convoluted and elongated with a strong zigzag pattern. These features were not seen in two normal controls nor in other patients with idiopathic DCM.

These very irregular and twisted intercalated disks were also seen in electron microscopy examinations of D-II-3 and C-VII-10 (Fig. 7). Note, we cannot exclude that modest structural changes in the insertion of sarcomeres

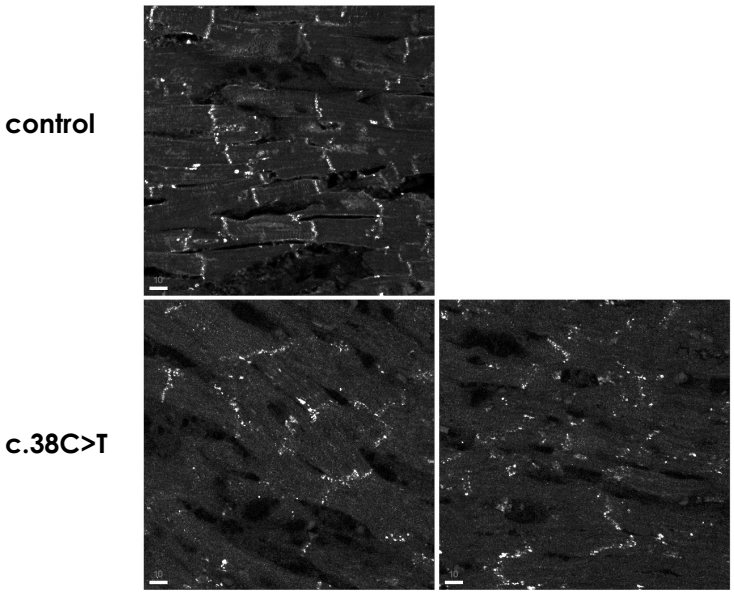

Figure 6. Connexin 43 distribution in a normal control and patient B-VI-9 with end-stage RV failure suggesting reduced amounts of Cx43. (color image: page 270)

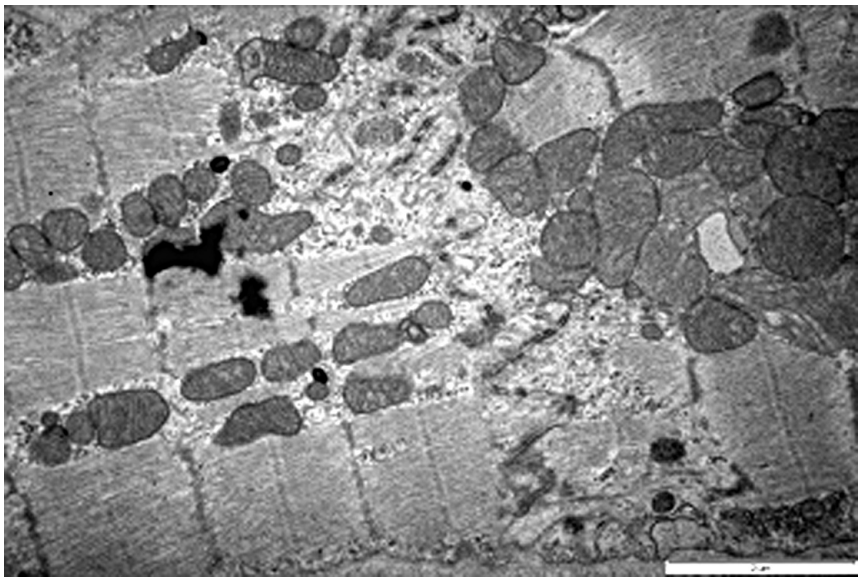

Figure 7: Electron microscopy of myocardial tissue of C-VII-10 showing irregular and bent intercalated disks with possibly some structural changes in the insertion at the disks. The Z-disks appear to be aligned, suggesting that the sarcomeres are in register.

at the disk are present, although this could also be an artifact. Notably, the Z-disks appeared to be aligned.

DISCUSSION

Disease-associated *DES* mutations are generally located in the a-helical domain of desmin and so far only five mutations in the N-terminal head domain have been described.[2,10,23,24] This head domain is believed to be important for the aggregation of tetramers to fibers (assembly) and the stability of the protein. Interaction with the outer cellular membrane occurs through the desmosome where desmin interacts with several proteins such as desmoplakin.[1,25] The assembly state of desmin seems to affect binding to desmoplakin, suggesting desmin has a role in the interaction with the desmosome.[26]

We recently described the index patients from families B and D in detail.[12] This identical mutation has also been identified in four patients from a two-generation Chinese family.[27] 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 were probably affected, and highlights additional immunohistochemical myocardial features.

Cardiac phenotype

Around 120 patients, carrying 53 different *DES* mutations have been described in different studies so far. In a recent review of desminopathy cases verified on a molecular level, cardiac involvement was found in 67 of 92 patients.[11] In our study of proven mutation or obligate carriers, we had sufficient data on 22 individuals who all had a cardiac phenotype: 16 initially presented with a pure cardiac phenotype, three with cardiac symptoms preceding neurological symptoms, and another three patients presented with a neurological picture before or simultaneously with cardiac pathology. We therefore conclude that the c.38C>T specific mutation is predominantly associated with cardiac pathology. This differs significantly from recently published data ($p<0.05$).[11]

Age of presentation: Details for 20 out of 27 patients were available on their age at initial presentation. The mean age at initial presentation was 35.5 years; 41.6 (27-54) years for females and 32.2 (14-53) years in males (non-significant). 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: seven DCM, five with either LV hypertrophy, diastolic dysfunction or an RCM-like phenotype, one with an unspecified form of cardiomyopathy, and two patients fulfilling ARVC task force criteria. The predominance of DCM agrees with a recent review which found DCM in 52 of 92 (57%) cases verified at a molecular level.[11] Our observation of patients fulfilling ARVC criteria has, however, not been reported before.

RV involvement: Apart from patient A-VI-5 and A-VI-7 fulfilling the ARVC task force criteria, four other mutation carriers (A-VI-2; B-VI-9; C-VII-8 and D-II-3) developed RV heart failure during the course of the disease or in its early stages. In the literature, right-sided heart failure has occasionally been reported.[28,29,30] This RV involvement was also evident from the presence of an RBBB in 10 patients, in whom it was sometimes the first or sole manifestation of early disease (A-VI-4; A-VII-4; B-IV-2; D-III-1; D-III-2 and E-II-2), or from ventricular tachycardias originating in the RV (A-VII-3, A-VI-7). Because desmin is highly expressed in conductive myocytes, it is easy to accept that conduction disorders, including RBBB, could be the first manifestation of a desminopathy.[31,32] This was also seen in our population, because 15 of 22 patients with data available, demonstrated an RBBB, LBBB or AV block at the time of initial presentation, suggesting that this 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 had died, had a transplant or had experienced appropriate ICD interventions at a mean age of 48.9 (27-63) years.

Neurological disease

In 9 of 15 patients in which neurological investigations were performed, a neurological disease was noticed, mainly affecting the lower limbs. The muscular weakness was proximally located in two patients and more distally in five others, suggesting a distal myopathy in some patients. A limb-girdle distribution was noticed in two patients. CK values were normal or only mildly elevated (in 9 and 7 patients, respectively). This has been recognized before, even in patients with generalized neurological disease.[3,10] CK measurement does not therefore necessarily help in diagnosing a desminopathy. The proportion of patients in our cohort who also demonstrated signs of a myopathy seems lower than the combined data from 92 desmin-mutation carriers (in which 80% of patients demonstrated a myopathy).[11]

Because other neurological diagnoses, such as a distal myopathy or LGMD, were made in some patients, we suggest that in patients with these diagnoses associated with cardiological symptoms, *DES* screening should be considered.

Histology-immunohistochemistry

The novel observation of frequent RV involvement in *DES* 38C>T mutation carriers, as shown by fulfilling ARVC task force criteria, RBBB and RV failure, and because desmosome cardiomyopathies are predominantly right-sided, suggests that the effect of the 38C>T might be due to an effect at the level of desmin-desmosome interactions. Other reasons to think of a role for desmin in RV heart failure due to an effect at the intercalated disk level were fueled by observations that *DES* null-mice demonstrated extensive RV involvement progressing after exercise[33,34,35] and because mice expressing a 7-amino-acid deletion of desmin also show reduced desmosomal and adherens junction proteins, including the major gap-junction protein connexin 43 (Cx43).[36] In Carvajal syndrome, a recessive cardiocutaneous syndrome caused by a homozygous desmoplakin deletion, highly convoluted intercalated disks with reduced levels of desmoplakin, plakoglobin and desmin signals were also found.[37] This suggests a common pathway in both desminopathies due to the 38C>T mutation and desmosome cardiomyopathies and might be 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 38C>T phenotype. However, all our patients studied demonstrated normal amounts of immunoreactive signal of the desmosomal proteins tested. This seems to underscore the notion that the desmin head domain is primarily involved in dimerization of the protein and generally does not affect mechanical coupling. However, the distribution pattern of desmosomal proteins was disrupted, leading to more convoluted and elongated intercalated disks with a zigzag appearance. This was confirmed in electron microscopy studies in C-VII-10 and D-II-3, who demonstrated irregular and bent intercalated disks with possibly some structural changes at the insertion of sarcomeres at the intercalated disks. As the myofibrils seemed aligned, the effect of this mutation in the head domain of DES might be due to a more localized misalignment of the 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 staggered.[38] Desmin null-mice also showed zigzag intercalated disks.[33] The observed changes in our patients were not identified in other patients with end-stage cardiac failure due to atherosclerosis or idiopathic DCM, or in normal controls, and this suggests it is related to this specific *DES* p.S13F cardiomyopathy (personal communication J.E. Saffitz).

In patient B-VI-9, a possible reduction of Cx43 was seen. 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.[36,37,39,40] In patient B-VI-9, however, other than the shape of the intercalated disks, the localization and distribution of desmosomal proteins was not affected. This mechanism is therefore unlikely to underlie reduced Cx43 and Cx43 down-regulation has to be considered as a result of the late-stage heart failure she demonstrated. This may underlie ventricular arrhythmias and hence sudden death, which is generally not an initial feature of disease in *DES* 38C>T mutation carriers.

Pathophysiology of the 38C>T mutation

The Ser13 residue is located in a highly conserved motif of desmin and is a phosphorylation site for protein kinase-C.[41] This protein-phosphorylation by protein kinases is believed to be important in the regulation of the organization of desmin and other intermediate filaments, and has been associated with desmin filament assembly and disassembly.[41,42] The results from experiments in 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.[42] In transfection studies it was found that, contrary to other *DES* mutations, the p.S13F mutant preserved its filamentous network.[27] These results and our observations indicate that no major misalignment of myofibrils occurs. The observed intercalated disk changes might therefore point towards another pathophysiology. It has been postulated that instability of the desmin filamentous network, or alternatively a mild or partial defect in network formation, could underlie the clinical picture with formation of a normal desmin network while leading to desmin accumulation.[27] Preliminary data on the functional aspects of head domain mutations of desmin have shown that during in vitro assembly, unit-length filament formation and elongation is conserved although abnormal structures were being found. This

was also found in combination with wild-type protein. Notably, in transfected cells some mutations distort regular arrangement of the exogenous and endogenous proteins.[43]

With respect to the pathophysiological mechanism of the p.S13F mutation, our observation that the mutation results in abnormalities at the intercalated disc region is important. It suggests that interactions between desmin filaments and components involved in intercalated disc 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 S13F mutation on the structure of desmin, interactions of other domains of the desmin molecule with such components are affected. In the latter case, the impairment of interactions with proteins of the plakin family, like desmoplakin, seems to be the most likely explanation for the observed effects.[26]

Conclusions

We have described 27 patients with a single-head-domain desmin mutation, the largest series of its kind so far. They demonstrate a highly variable, yet predominantly cardiological clinical picture with, in particular, right-sided myocardial involvement (including the diagnosis of ARVC), RBBB and/or VTs originating from the RV in the early phases of the disease. Males manifest disease at an earlier age than females. Some of the families showed hardly any neurological disease. Immunohistochemistry demonstrated an effect of the mutated protein on the architecture of the regions where junctional proteins reside; we found more convoluted and elongated intercalated discs. This suggests 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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