ORIGINAL ARTICLE

Desmosomal protein gene mutations in patients with idiopathic dilated cardiomyopathy undergoing cardiac transplantation: a clinicopathological study

Pablo Garcia-Pavia,[1] Petros Syrris,[2] Clara Salas,[3] Alison Evans,[2] Jesus G Mirelis,[1] Marta Cobo-Marcos,[1] Carlos Vilches,[4] Belen Bornstein,[5] Javier Segovia,[1] Luis Alonso-Pulpon,[1] Perry M Elliott[2]

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[1]Cardiomyopathy Unit, Heart Failure and Heart Transplant Section, Department of Cardiology, Hospital Universitario Puerta de Hierro, Madrid, Spain
[2]Inherited Cardiovascular Disease Unit, The Heart Hospital, University College London, London, UK
[3]Department of Pathology, Hospital Universitario Puerta de Hierro, Madrid, Spain
[4]Department of Immunology, Hospital Universitario Puerta de Hierro, Madrid, Spain
[5]Department of Biochemistry, Hospital Universitario Puerta de Hierro, Madrid, Spain

Correspondence to
Dr Pablo Garcia-Pavia, Cardiomyopathy Unit, Department of Cardiology, Hospital Universitario Puerta de Hierro, Manuel de Falla 2, 28222 Madrid, Spain; pablogpavia@yahoo.es

Accepted 28 June 2011
Published Online First 22 August 2011

ABSTRACT

Backgroud Idiopathic dilated cardiomyopathy (DCM) is the most frequent indication for orthotopic heart transplantation. It has been suggested that mutations in genes encoding desmosomal proteins, more typically associated with arrhythmogenic right ventricular cardiomyopathy, are a cause of DCM.

Objectives To determine the frequency of desmosomal protein gene mutations in heart transplant recipients and their families and to examine histopathological characteristics of explanted organs from mutation carriers.

Methods 89 unrelated patients aged 47.9±13.5 years (80% male) transplanted for end-stage DCM underwent genetic screening of five desmosomal genes (PKP2, DSP, DSC2, DSG2 and JUP). The findings were correlated with clinical features and histological characteristics in explanted hearts.

Results Pathogenic mutations were identified in 12 patients (13%). Five additional patients (6%) had genetic variants of unknown significance. The clinical phenotype of patients with pathogenic mutations was indistinguishable from that observed in patients without mutations. Evaluation of 76 relatives from 14 families with sequence variants (11 with pathogenic mutations and three with variants of unknown effect) identified 38 mutation carriers, four of whom had an overt DCM phenotype. Evidence of co-segregation of mutations with DCM phenotype was found in five families. Histological evaluation of explanted hearts did not show any specific features in patients with pathogenic mutations.

Conclusions Mutations in desmosomal genes are frequent in patients with advanced DCM undergoing cardiac transplantation. These findings emphasise the importance of familial evaluation and genetic counselling in patients with end-stage DCM and pose important challenges for current histopathological criteria for arrhythmogenic right ventricular cardiomyopathy.

INTRODUCTION

Idiopathic dilated cardiomyopathy (DCM), defined as dilation and systolic impairment of the left or both ventricles in the absence of hypertension, coronary artery disease or valvular abnormalities,[1] is the commonest cause of heart failure in the young[2] and the most frequent indication for orthotopic heart transplantation.[3] Family studies in predominantly stable outpatient populations suggest that up to 48% of patients have a familial predisposition to disease.[4 5] However, the genetic basis of disease of most patients is still unknown,[6 7] particularly in those with advanced disease in whom there are few genetic or family studies.

A recent study has suggested that at least 5% of patients with chronic stable DCM harbour mutations in genes encoding desomosomal proteins.[8] The primary aim of this study was to determine the frequency of pathogenic mutations in desmosomal protein genes in heart transplant recipients and their families and to compare and contrast the histopathological characteristics of explanted organs and clinical features of patients with and without mutations.

METHODS

Study population

Ninety-five patients undergoing heart transplantation for DCM were randomly selected from a cohort of 187 patients transplanted for DCM at our institution between September 1993 and December 2007 (39% of transplants over that period). Only those patients fulfilling the WHO/International Society and Federation of Cardiology Task Force clinical criteria for DCM[9] at the time of heart transplantation (ie, left ventricular end-diastolic diameter >27 mm/m^2 and an ejection fraction <40% or fractional shortening <25% in the absence of abnormal loading conditions, coronary artery disease, congenital heart lesions and other systemic diseases) were included. Patients with significant coronary artery disease at pathological examination of the explanted heart were excluded.

Pretransplant assessment comprised physical examination, 12-lead ECG, echocardiography, 24 h ambulatory ECG monitoring, 6 min walk test, upright exercise testing, biventricular radionuclide ventriculography and cardiac catheterisation. Additional studies including endomyocardial biopsy, electrophysiological study and cardiac MRI were performed only if there was a clinical indication to do so.

Patient records were reviewed retrospectively by two physicians blinded to the genetic results (PG-P, JGM). Clinical data and results from the first pretransplant evaluation at our unit were collected. No patient had myocarditis (focal or diffuse inflammatory lymphocyte infiltration in the interstitium

with myocyte damage/necrosis) on pretransplant endomyocardial biopsy.

Based on patient history and family pedigree analysis, DCM was defined as familial if one or more relatives (in addition to the proband) had DCM during life or at post-mortem examination or had experienced unexplained sudden cardiac death before the age of 35 years.[10]

Genetic evaluation

Since September 1993 all patients placed on the heart transplant waiting list at our centre have been asked to provide a blood sample for genetic analysis. DNA from these samples is extracted and stored at −70°C. In this study, DNA samples were amplified by PCR as described previously[11–13] using primers designed to amplify the coding exons and the flanking intronic sequences of five arrhythmogenic right ventricular cardiomyopathy (ARVC)-related desmosome-encoding genes: plakophillin-2 (PKP2), desmoplakin (DSP), desmocollin-2 (DSC2), desmoglein (DSG2) and plakoglobin (JUP). Following PCR amplification, direct sequencing of amplicons was performed on an ABI PRISM 3130 DNA analyser using BigDye Terminator chemistry (V.3.1, Applied Biosystems, Warrington, UK). Primer sequences and PCR conditions are available on request.

For every sequence variant detected in patients with DCM, a cohort of 200 ethnically-matched control subjects was screened using the same methods. Every sequence variant found was cross-referenced to the international ARVC database.[14] Patients were classified as carriers of pathogenic mutations if they had a genetic variant that was reported in the database as pathogenic, a novel sequence variant not found in controls that predicts a premature truncation, frameshift or abnormal splicing, a novel missense mutation that affects a conserved amino acid residue and co-segregated with disease on familial evaluation, or a variant classified in the international database as a variant of unknown effect which co-segregated with disease on subsequent family screening.

Patients with sequence variants classified in the international database as variants of unknown effect or with novel missense mutations not found in controls without corroborative family screening data were considered as carriers of mutations of uncertain significance.

The likelihood of pathogenic effect of missense sequence variants in desmosomal genes was determined by four in silico prediction methods: the Grantham score, PolyPhen, PolyPhen-2 and SIFT (see references w1–w4 in online supplement). The Grantham score method uses a formula for the difference between amino acids according to their physicochemical properties with high values indicating radical mutations. PolyPhen predicts the possible effect of amino acid substitutions based on physiochemical differences, evolutionary conservation and the proximity of the substituted amino acid to important known or predicted functional protein domains. SIFT predicts the functional importance of amino acid changes based on the alignment of orthologous and/or paralogous protein sequences. A missense sequence change is most likely to be pathogenic when it is classified as 'probably damaging' by PolyPhen or 'not tolerated' by SIFT. Conservation of amino acid residues in desmosomal genes was determined by Homologene (http://www.ncbi.nlm.nih.gov/homologene) by multiple alignment of orthologues in various species including *Homo sapiens*, *Pan troglodytes*, *Mus musculus*, *Rattus norvegicus*, *Gallus gallus*, *Bos taurus*.

Family screening

All relatives of patients with pathogenic mutations or variants of uncertain significance were offered clinical and genetic evaluation after genetic counselling. Clinical evaluation included physical examination, ECG and an echocardiogram. In accordance with our unit's genetic testing policy, genetic screening was not offered to relatives <16 years of age if they were asymptomatic and clinical screening tests were completely normal.

Family screening was considered positive if one or more relatives had DCM and the same genetic defect as the proband.

Pathological examination of explanted hearts

Gross (non-formalin-fixed) and microscopic examinations of explanted hearts were performed as described previously[15] by a histopathologist (CS) blinded to the genetic and clinical data. Blocks of the anterior and posterior free wall of the left ventricle, right ventricle and interventricular septum were removed systematically for histopathological study. Specimens were also taken from areas showing macroscopic abnormality. Tissue samples were stained with H&E, orcein and Masson's trichrome. The presence of fibrosis and fatty tissue was evaluated in three preparations per ventricle. The average extent of fibrosis and fatty tissue from epicardium to endocardium in both ventricles was recorded (<10%, 10–25%, 25–50%, 50–75% and >75%). Isolated epicardial fatty infiltration was excluded from the analysis. Other microscopic abnormalities including disorganised myocardial cells, degenerative myocytes and inflammatory infiltrates were noted.

Statistical analysis

Continuous variables are expressed as mean±SD values. Discrete variables are shown as percentages. Differences between means were compared using the Student t test and the Mann–Whitney U test for normally distributed and non-normally distributed continuous data, respectively. χ^2 and Fisher exact analysis were used to test for associations between dichotomous variables. Probability values reported are two-sided and values <0.05 were considered statistically significant. All data were analysed using the SPSS software V.5.0.

RESULTS

Following a review of case notes, six of the 95 patients were excluded for the following reasons: family relation (brother) to another selected patient (n=1); past history of chemotherapy (n=1); history of toxic oil syndrome (n=1); DCM associated with muscular dystrophy known to be caused by a dystrophin mutation (n=1); and dilated phase of restrictive cardiomyopathy (n=2). The final study cohort therefore comprised 89 patients (mean age at transplantation 49.2±13.1 years, range 12.3–68.6, 80% male). All patients were Caucasians. Their clinical characteristics are summarised in table I in the online supplement. Forty-three patients (48%) were evaluated as inpatients, 10 (11%) on the intensive care unit. Two patients were removed from the heart transplant waiting list due to improvement in their functional class but were subsequently relisted and transplanted 248 and 378 days later, respectively. Before transplant, none of the patients fulfilled current diagnostic criteria for ARVC[16 17] or had a family history of ARVC.

Sixteen patients (18%) had a known family history of DCM and seven (8%) had a family history of sudden cardiac death (only one in a relative aged <35 years). From history and pedigree analysis, 17 patients (19%) fulfilled familial DCM criteria.[10]

Genetic analysis

Twelve patients (13%) had pathogenic mutations and five patients (6%) had genetic variants of unknown pathogenicity.

Table 1 Clinical and genetic characteristics of probands with genetic variants in desmosomal genes

Pt	Age (years)	Sex	Mutation	Coding effect	NYHA	ECG	Holter	LV and RV	Exercise test
A. Probands with pathogenic mutations									
D1.1	62	M	PKP2—c.1759G>A DSC2—c.907G>A*	V587I V303M	IV	Atrial fibrillation. QRSd: 100 ms. Negative T waves V5, V6, I and aVL.	—	LVedD: 70 mm; LVedD: 151%. LVEF: 28% RVEF†: 36%	3.6 METSVE during recovery
D2.1	37	F	DSP—c.2720G>A* DSP—c.5513G>A	R907H R1838H	IV	Sinus rhythm. QRSd: 60 ms. Negative T waves V5, V6	—	LVedD: 44 mm; pLVedD: 99%. LVEF: 18%. Impaired RV function	—
D3.1	52	M	PKP2—c.419C>T	S140F	III	Atrial fibrillation. Paced ventricular rhythm	Isolated VE	LVedD: 70 mm; pLVedD: 153%. LVEF: 31%. RV dilation. RVEF†: 20%	—
D5.1	50	M	JUP—c.56C>T	T19I	II	Atrial fibrillation. QRSd: 80 ms. Negative T waves V4, V5, V6, II, III and aVF	NSVT	LVedD: 55 mm; pLVedD: 113%. LVEF: 43%. RVEF†: 45%.	7 METS No arrhythmias
D8.1	56	M	DSG2—484delG	M162fsX171	III	Atrial fibrillation. Paced ventricular rhythm	—	LVedD: 69 mm; pLVedD: 144%. LVEF: 25%. RV dilated with impaired function	—
D9.1	19	M	PKP2—c.1759G>A	V587I	II	Sinus rhythm. QRSd: 90 ms. Negative T wave V6	Isolated VE	LVedD: 76 mm; pLVedD: 175%. LVEF: 25%. Normal RV	6.6 METS No arrhythmias
D11.1	60	M	DSP—c.6881C>G	A2294G	II	Sinus rhythm. QRSd: 160 ms. LBBB	No arrhythmias	LVedD: 86 mm; pLVedD: 178%. LVEF: 25%. RVEF†: 27%	1.8 METS No arrhythmias
D12.1	45	M	DSG2—c.1003A>G DSP—c.5498A>T*	T335A E1833V	IV	Atrial fibrillation. Paced ventricular rhythm.	NSVT	LVedD: 70 mm; pLVedD: 139%. LVEF: 32%. Normal RV	—
D16.1	35	F	DSP—c.8290_8291 insTGCT	insA2765fsX2787	II	Sinus rhythm. QRSd: 110 ms. Negative T waves V5, V6, I, aVL and V2	—	LVedD: 67 mm; pLVedD: 152%. LVEF: 21%. Normal RV	5.6 METS VE on exercise
D17.1	33	M	DSG2—c.1174G>A DSC2—c.2194T>G*	V392I L732V	IV	Sinus rhythm. QRSd: 90 ms. Negative T waves V1, V2, V5, V6 and aVL	—	LVedD: 71 mm; pLVedD: 159%. LVEF: 27%. RV dilated with impaired function	—
D18.1	51	M	DSP—c.6881C>G	A2294G	III	Sinus rhythm. QRSd: 130 ms. LBBB	—	LVedD: 83 mm; pLVedD: 179%. LVEF: 27%. RV dilation	—

Continued

Table 1 Continued

Pt	Age (years)	Sex	Mutation	Coding effect	NYHA	ECG	Holter	LV and RV	Exercise test
D19.1	32	F	PKP2—c.1759G>A	V587I	II	Sinus rhythm. QRSd: 80 ms. Negative T waves in V1	NSVT	LVedD: 71 mm; pLVedD: 156%. LVEF: 20% RV dilation	8.8 METS No arrhythmias
B. Probands with desmosomal gene variants of unknown effect									
D6.1	64	F	DSC2—c.266C>T	S89L	III	Sinus rhythm. QRSd: 120 ms. LBBB	NSVT	LVedD: 70 mm; pLVedD: 151%. LVEF: 25%. Normal RV	—
D7.1	61	M	DSP—c.8461C>T	S2821L	II	Atrial fibrilation. QRSd: 120 ms. LBBB	No arrhythmias	LVedD: 68 mm; pLVedD: 147%. LVEF: 25%. RV dilated	2.2 METS No arrhythmias
D13.1	50	M	PKP2—c.184C>A	Q62K	II	Sinus rhythm. QRSd: 90 ms. Negative T wave II and aVF	NSVT	LVedD:58 mm; pLVedD: 118%. LVEF: 40%. RVEF†: 37%	6.7 METS. VE on exercise and recovery
D15.1	48	F	DSG2—c.3295A>G	T1099A	II	Sinus rhythm. QRSd: 85 ms. Negative T wave 5, V6, I, aVL, II and aVF	—	LVedD: 58 mm; pLVedD: 123%. LVEF: 20%. RVEF†: 36%	4.6 METS No arrhythmias
D20.1	56	M	JUP—c.526C>T	R176C	III	Sinus rhythm. QRSd: 115 ms. Negative T wave V5, V6, I, and aVL	No arrhythmias	LVedD: 83 mm; pLVedD: 180%. LVEF: 21%. RVEF†: 45%	—

*Unknown variant.
†RV function measured by RV isotopic ventriculography.
F, female; LBBB, left bundle branch block; LV, left ventricle; LVedD, left ventricle end-diastolic dimension; M, male; NSVT, non-sustained ventricular tachycardia; NYHA, New York Heart Association Class; Pt, patient; pLVedD, predicted left ventricle end-diastolic dimension as calculated by Henry *et al*[18]; QRSd, QRS duration; RV, right ventricle; VE, ventricular ectopy.

Table 2 Clinical, familial and histological characteristics of probands with genetic variants in desmosomal genes

Pt	Family	Clinical course prior to transplant	Histology	ARVC criteria
A. Probands with pathogenic mutations:				
D1.1	Sister with DCM dead age 46	Palpitations since age 55. Transplanted urgently due to refractory HF. Days since first evaluation:12. Days on WL: 12	RV: absence of fibrosis or fatty infiltration. LV: fibrofatty infiltration (10–25% of wall thickness)	0 major 2 minor (repolarisation, depolarisation)
D2.1	Mother with DCM age 64	Presented with cardiogenic shock. BiVAD as bridge to HT. Transplanted urgently. Days since first evaluation: 10. Days on WL: 6	RV: fatty infiltration (25–50% of wall thickness). LV: fibrosis infiltration (<10% of wall thickness). Myocyte degeneration and lymphocyte infiltration	0 major 1 minor (repolarisation)
D3.1	–	Chronic HF. Days since first evaluation: 637. Days on WL: 514	RV: absence of fibrosis or fatty infiltration. LV: fibrosis (<10% of wall thickness). Signs of myocyte degeneration	0 major 0 minor
D5.1	Father with DCM, SCD age 53.	Chronic HF. Transplanted urgently due to deterioration while being on WL. Days since first evaluation: 1371. Days on WL: 88	RV: fibrosis (<10% of wall thickness). LV: fibrosis (10–25% of wall thickness)	0 major 1 minor (arrhythmia)
D8.1	–	Chronic HF. Days since first evaluation: 483. Days on WL: 457	RV: fibrosis (<10% of wall thickness). LV: fibrosis (10–25% of wall thickness) Myocyte degeneration and lymphocyte infiltration.	0 major 0 minor
D9.1	–	Acute HF. Transplanted urgently. Days since first evaluation:54. Days on WL: 43	RV: fibrofatty infiltration (<10% of wall thickness). LV: fibrosis (<10% of wall thickness). Signs of myocyte degeneration	0 major 1 minor (repolarisation)
D11.1	–	Chronic HF NSVT in successive holters. Elective HT. Days since first evaluation: 667. Days on WL: 245	RV: fibrofatty infiltration (10–25% of wall thickness). LV: fibrosis (10–25% of wall thickness). Myocyte degeneration.	0 major 0 minor
D12.1	Brother with DCM age 52	Acute HF. Transplanted urgently. Days since first evaluation:10. Days on WL: 10	RV: fibrofatty infiltration (10–25% of wall thickness). LV: fibrosis (10–25% of wall thickness). Biventricular lymphocyte infiltration	0 major 1 minor (arrhythmia)
D16.1	Sister with DCM age 45	Chronic HF. VF while being under revaluation for HT. Transplanted urgently after SCD due to cardiogenic shock. Days since first evaluation: 392. Days on WL: 3	RV: fibrosis (25–50% of wall thickness). LV: fibrosis (10–25% of wall thickness). Myocyte degeneration.	0 major 2 minor (repolarisation, depolarisation)
D17.1	Mother DCM died age 38. Sister DCM at age 34	Chronic HF Transplanted urgently after acute deterioration while waiting for HT evaluation. Days since first evaluation: 7. Days on WL: 2	RV: absence of fibrosis or fatty infiltration. LV: absence of fibrosis or fatty infiltration	0 major 1 minor (repolarisation)
D18.1	Brother with DCM age 58	Chronic HF Days since first evaluation: 163. Days on WL: 149	RV: absence of fibrosis or fatty infiltration. LV: absence of fibrosis or fatty infiltration. Signs of myocyte degeneration. Lymphocyte infiltrates on RV	0 major 0 minor
D19.1	–	Chronic HF Transplanted urgently after acute deterioration. Days since first evaluation: 3781. Days on WL: 1	RV: absence of fibrosis or fatty infiltration. LV: fibrosis (<10% of wall thickness)	0 major 1 minor (arrhythmia)
B. Probands with desmosomal gene variants of unknown effect				
D6.1	–	Chronic HF Elective HT Days since first evaluation: 847. Days on WL: 842	RV: fatty infiltration (25–50% of wall thickness). LV: fibrosis (<10% of wall thickness)	0 major 1 minor (arrhythmia)

Continued

Table 2 Continued

Pt	Family	Clinical course prior to transplant	Histology	ARVC criteria
D7.1	Twin brother dead age 55 after falling from a roof	Chronic HF Elective HT Days since first evaluation: 45. Days on WL: 26	RV: fibrofatty infiltration (10–25% of wall thickness). LV: fibrosis (<10% of wall thickness) Myocyte degeneration	0 major 0 minor
D13.1	–	Chronic HF. VT 10 years before HT when LVEF was 50% Days since first evaluation: 1585. Days on WL: 317	RV: fibrosis (25–50% of wall thickness). LV: fibrosis (10–25% of wall thickness). Myocyte degeneration	0 major 1 minor (arrhythmia)
D15.1	–	Chronic HF Elective HT. Days since first evaluation: 185. Days on WL: 25	RV: fibrofatty infiltration (<10% of wall thickness). LV: absence of fibrosis or fatty infiltration	0 major 1 minor (repolarisation)
D20.1	–	Chronic HF. Elective HT. Days since first evaluation: 61. Days on WL: 51	RV: fatty infiltration (25–50% of wall thickness). LV: fibrosis (10–25% of wall thickness)	0 major 2 minor (repolarisation, depolarisation)

HF, heart failure; HT, heart transplant; LV, left ventricle; Pt, patient; RV, right ventricle; SCD, sudden cardiac death; VF, ventricular fibrillation; VT, ventricular tachycardia; WL, waiting list.

None of these genetic abnormalities were found in the 200 control patients.

Patients with pathogenic mutations

Family evaluation was possible in 11 of the 12 patients with pathogenic mutations. Seven patients had mutations that had been described previously in patients with ARVC[14]; two had novel mutations in DSG2 and DSP that predicted premature truncation of the transcribed protein; three patients (D2, D11 and D18) had missense mutations in DSP (D11 and D18 had the same mutation previously classified as an unknown variant and D2 had two novel mutations) that co-segregated with DCM in families D2 and D18. Degree of conservation through species, current classification in ARVC international database and predicted pathogenicity at in silico analysis of missense mutations found are shown in table II in the online supplement.

Co-segregation of mutations with DCM in another relative was demonstrated in five families (D2, D5, D12, D16, and D18; see family trees in online supplement). Three patients (D1, D12 and D17) with pathogenic mutations also carried sequence variants of unknown significance.

Tables 1 and 2 show the clinical course, genetic, pathological and family data for patients with pathogenic mutations. No significant differences in clinical and echocardiographic characteristics were found between mutation carriers and patients without pathogenic genetic variants (table 3).

Patients with genetic variants of unknown effect

Five patients (6%) had genetic variants of unknown pathogenicity. Four patients (D6, D7, D15 and D20) had previously unreported missense mutations that were not found in controls. Another patient (D13) had a previously described mutation in PKP2 classified as a genetic variant of unknown effect in the ARVC/D mutation database.[14] All these missense mutations affect amino acid residues conserved through species (see table III in online supplement). Family evaluation was either negative (D6, D7, D20) or unavailable (D13, D15).

Tables 2 and 3 show the clinical course, genetic, pathological and familial information for patients with variants of unknown effect.

Family evaluation

Of a total of 17 families with genetic abnormalities, 14 (82%) agreed to participate in the study. From a total of 85 relatives contacted, 76 (89%) agreed to be screened. Thirty-eight relatives (50%) were carriers of the same genetic abnormalities as those found in their families (27 had pathogenic mutations and 11 had variants of unknown significance). Five relatives were found to have impaired left ventricular function. In one of them (patient D5.2) this was caused by coronary artery disease. In the remaining four relatives with DCM, coronary artery disease was excluded by coronary angiography. None of them fulfilled old or current ARVC criteria (see table IV in online supplement). The penetrance of DCM among mutation carriers was therefore 14% (four affected individuals from 27 mutation carriers). Echocardiographic and ECG characteristics of unaffected relatives were indistinguishable between those who were carriers of genetic abnormalities and those who were not, except for T wave inversion in lead III which was found more frequently among mutation carriers (see table V in online supplement).

Histopathological findings

Fifty-eight explanted hearts (65%) showed fatty (n=35; 39%) and/or fibrotic infiltration (n=45; 50%) in the right ventricle and 72 (81%) had fat (n=7; 8%) and/or fibrosis (n=71; 80%) in the left ventricle. The percentage of fibrofatty change was highly variable with only 12 (14%) and 12 (14%) patients having more than 25% of their right ventricle and left ventricle walls occupied, respectively. Twenty explants (23%) had lymphocyte infiltrates and 39 (44%) had signs of active myocyte degeneration.

Among probands with mutations, findings in explanted hearts were very heterogeneous (table 2) with some showing extensive fibrofatty infiltration and others a complete absence of fat and fibrotic tissue (figure 1). Patients with mutations of uncertain effect also exhibited a wide range of fibrofatty infiltration (table 2).

Differences between both groups in the amount of left ventricular and right ventricular walls occupied by fat and/or fibrosis were not statistically significant (table 3).

DISCUSSION

This study shows that a substantial proportion of patients undergoing heart transplantation for end-stage DCM have mutations in genes coding for desmosomal proteins. Importantly, clinical findings prior to transplantation and histological evaluation of explanted hearts in mutation carriers were similar to those observed in non-mutation carriers. A wide spectrum of histological findings was noted among mutation carriers and patients with mutations of uncertain significance.

Table 3 Clinical, electrocardiographic, echocardiographic and histological characteristics of patients with and without desmosomal protein gene mutations

Variable	Desmosomal mutations group (n=12)	Non-carrier group (n=72)	p Value
Mean age (years)	44.8±13.1	48.2±13.3	0.42
Sex, n (%)			0.87
Male	9 (75%)	59 (82%)	
Female	3 (25%)	13 (18%)	
NYHA class, n (%)			0.25
I	0 (0%)	1 (1%)	
II	5 (42%)	12 (17%)	
III	4 (33%)	36 (50%)	
IV	3 (23%)	23 (32%)	
Family history			
Dilated cardiomyopathy, n (%)	5 (42%)	11 (15%)	0.08
Sudden cardiac death, n (%)	1 (8%)	6 (8%)	1
History of sustained VT, n (%)	0 (0%)	5 (7%)	1
Transplant			
Urgent HT, n (%)	7 (58%)	25 (35%)	0.22
Time on waiting list (days)	128±183	101±181	0.64
Time since first evaluation (days)	641±1066	309±411	0.31
ECG			
Sinus rhythm, n (%)	7 (58%)	53 (74%)	0.46
Atrial fibrillation, n (%)	5 (42%)	19 (26%)	0.46
Mean QRS duration, (ms)*	100±30	117±35	0.17
Left bundle branch block, n (%)	2 (17%)	24 (33%)	0.41
Right bundle branch block, n (%)	0 (0%)	1 (1%)	1
Non-specific bundle branch block, n (%)	0 (0%)	9 (13%)	0.35
Paced rhythm, n (%)	3 (25%)	10 (14%)	0.58
Terminal activation QRS V1–V3 >55 ms, n (%)†	2 (28%)	4 (11%)	0.55
T wave inversion, n (%)†			
V1	2 (29%)	2 (6%)	0.24
V2	1 (14%)	1 (3%)	0.75
V3	0 (0%)	3 (9%)	1
V4	1 (14%)	3 (9%)	1
V5	5 (71%)	17 (49%)	0.49
V6	6 (86%)	20 (57%)	0.32
I	2 (29%)	8 (23%)	1
aVL	3 (43%)	12 (34%)	1
II	2 (29%)	7 (20%)	1
III	1 (14%)	7 (20%)	1
aVF	1 (14%)	7 (20%)	1
Arrhythmia during exercise test‡			
Ventricular ectopy on exercise, n (%)	1 (17%)	10 (21%)	1
Ventricular ectopy during recovery, n (%)	1 (17%)	16 (34%)	0.69
24-h ECG monitoring§			
Non-sustained VT, n (%)	3 (50%)	25 (54%)	1
Echocardiography			
LVedD (mm)	69±11	71±11	0.70
Predicted LVedD (%)	150±24	151±23	0.46
LVEF (%)	27±7	25±8	0.46
LV wall motion abnormalities, n (%)	1 (8%)	5 (7%)	1
RV dilation, n (%)	4 (33%)	31 (44%)	0.53
RV systolic impairment, n (%)	6 (50%)	21 (29%)	0.27
RV wall motion abnormalities	0 (0%)	3 (4%)	1
RVEF on isotopic ventriculography (%)¶	29±10	25±11	0.48
Patients with RVEF by isotopic ventriculography < LVEF by echocardiography, n (%)¶	2 (33%)	15 (44%)	0.96
Histopathology			
LV fatty infiltration, n (%)	1 (8%)	6 (8%)	1
RV fatty infiltration, n (%)	4 (33%)	28 (39%)	0.96
LV fibrotic infiltration, n (%)	10 (83%)	57 (79%)	0.94
RV fibrotic infiltration, n (%)	6 (50%)	36 (50%)	1
LV fibrotic or fibrofatty infiltration >25%, n (%)	0 (0%)	12 (17%)	0.28
RV fibrotic or fibrofatty infiltration >25%, n (%)	2 (16%)	7 (10%)	0.83
RV fibrotic or fibrofatty infiltration >50%, n (%)	0 (0%)	5 (7%)	1

*Patients with paced ventricular rhythm excluded.
†Patients with bundle branch block or paced ventricular rhythm excluded.
‡Six patients (50%) from the mutated group and 47 (65%) from the non-carrier group underwent exercise test.
§Six patients (50%) from the mutated group and 46 (64%) from the non-carrier group underwent 24h ECG monitoring.
¶Six patients (50%) from the mutated group and 34 (49%) from the non-carrier group underwent RV isotopic ventriculography.

Some patients exhibited extensive fibrofatty infiltration while others showed near normal histology.

Role of desmosomal genes in DCM

Desmosomes are secondary mechanical intercellular junctions present in abundance in epithelial tissues and the myocardium. Together with the adherens and gap junctions, the desmosomes connect myocardial cells and maintain the mechanical and electrical integrity of the heart.[19] The desmosomes have a complex structure that includes adhesion molecules of the cadherin family (desmogleins and desmocollins) and proteins of the plakin and catenin families (desmoplakin, plakophilins, and plakoglobin) which link intermediate filaments of the cytoskeleton to the desmosomal cadherins.[20] Some desmosomal proteins (particularly plakoglobin) act as nuclear signalling molecules via Wnt signalling pathways.[21] A link between mutations in desmosomal protein genes and ARVC was first suggested by the discovery of homozygous mutations in plakoglobin (Naxos disease) and DSP (Carvajal syndrome).[22] Subsequent studies demonstrated mutations in these and other desmosomal proteins in patients with the more common (but phenotypically more variable) autosomal dominant form of the disease.[23] While the defining feature of ARVC is involvement of the right ventricle, it was recognised quite early that the left ventricle (in particular the posterolateral wall) can also be involved and that progressive dilation of both ventricles can lead to an end stage indistinguishable from DCM. However, until recently DCM has been thought to be a distinct clinical entity unrelated in most instances to ARVC. The findings in this study suggest that the rigid distinction between DCM and ARVC is to some extent arbitrary. For example, many of the mutations reported in this study have been reported in patients with a pure ARVC phenotype, but none of the patients had clinical features that fulfil recently modified criteria for the diagnosis of ARVC.[17] Although biventricular fibrosis was common, histological analysis of explanted hearts revealed fibrofatty change in both ventricles in only two patients (2%) of the total study cohort and, interestingly, there were no differences in conventional histology in patients with and without pathogenic mutations.

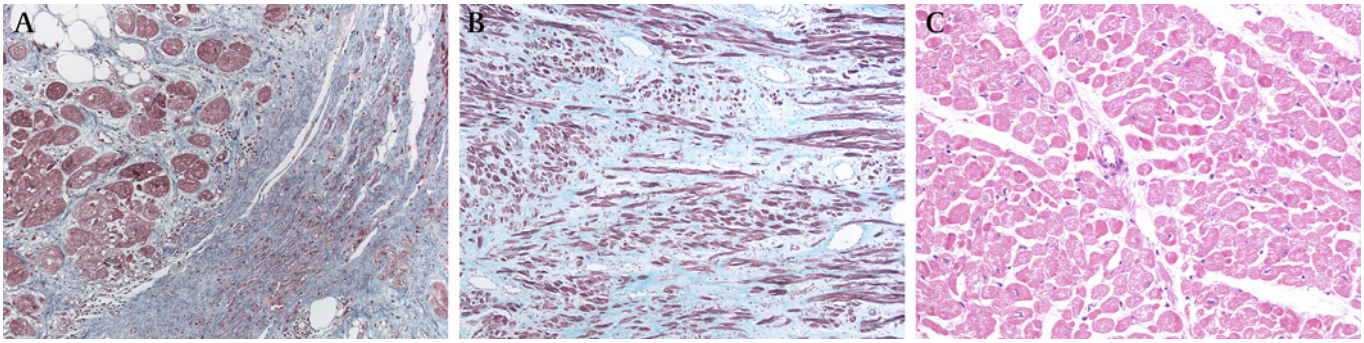

Figure 1 Microscopic examinations from patients (A) D12, (B) D19 and (C) D17. (A) Masson's trichrome stain (×25) showing fibrofatty infiltration of the right ventricle. (B) Masson's trichrome stain (×200) showing extensive fibrotic replacement of myocytes in the left ventricle. (C) Haematoxylin-eosin stain (×200) showing absence of fibrofatty infiltration with normal disposition of myocytes in the left ventricle.

Previous studies in transplant populations

Very few studies have examined genetic characteristics in heart transplant recipients.[24] Monserrat *et al*[25] undertook clinical and echocardiographic evaluation in relatives of 43 heart-transplanted patients with DCM and found that DCM was familial in 11 probands (25.6%) and possibly familial in a further 11 individuals (25.6%). The pattern of inheritance was autosomal dominant in most families and some relatives had other genetically determined cardiac diseases such as hypertrophic cardiomyopathy. Karkkainen *et al*[24] investigated the prevalence of lamin A/C mutations (so far the most frequent gene associated with familial DCM) among 66 heart transplant recipients and found mutations that explained DCM in six cases (9%). Family screening in our study revealed two relatives (D12.3 and D16.2) with previously unknown (and untreated) DCM. This, along with the demonstration that 50% (7/14) of the screened families and 19% of the entire cohort had evidence of familial DCM, illustrates the importance of genetic counselling and the provision of clinical screening to the relatives of patients with end-stage DCM.

Clinical implications

Although the genetic basis of DCM has been known for more than 25 years[26] and mutations in more than 40 genes have been associated with the condition,[6 7] the use of genetic screening in daily practice has been limited by the low yield of current testing strategies. When systematic genetic screening has been applied to large DCM cohorts, a significant number of mutations (around 5%) has been found only in two genes (LMNA and MYH7).[27 28] More recently, screening of 14 genes associated with DCM in a cohort of 312 patients with DCM has shown that approximately 27% of DCM probands had possible or likely disease-causing genetic variants.[27 29 30]

While our study suggests that the addition of desmosomal protein gene mutations to the standard panel of tests offered to patients with DCM has the potential to increase the efficiency of genetic testing, low clinical penetrance and emerging data showing that variants in desmosomal genes are relatively common in some populations[31] means that caution should be employed in the interpretation of mutation analysis in this context. Compound heterocigosity (multiple mutations in ≥2 genes) is increasingly identified in other inherited cardiomyopathies, and it is highly probable that this phenomenon will affect the clinical expression of a disease like DCM where so many genes have been linked with the condition. As it is possible that some genetic variants merely increase susceptibility to disease or modify the response to other genetic, epigenetic or environmental factors, testing for desmosomal protein gene mutations should always be performed in conjunction with careful clinical phenotyping and familial evaluation.

CONCLUSIONS

Desmosomal protein gene mutations are common in patients with end-stage DCM but are not associated with a specific clinical or histological phenotype. These findings emphasise the importance of genetic counselling for patients undergoing cardiac transplantation and pose important challenges for current histopathological criteria for ARVC.

Acknowledgements We are indebted to the patients and their families for their participation in the study. We also gratefully acknowledge Mrs Ana Briceño for her excellent technical assistance and Drs Francisco Hernández, Inés Garcia, Manuel Sánchez and Patricia Avellana for their valuable help in approaching the families.

Funding This work was partially supported by the Instituto de Salud Carlos III (grant PI08/0978) and the Spanish Ministry of Health (Red Cooperativa de Insuficiencia Cardiaca (REDINSCOR) (RD06/03/0018). Some of this work was undertaken at UCLH/UCL who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme.

Competing interests None.

Ethics approval This study was conducted with the approval of the Hospital Universitario Puerta de Hierro and complies with the principles of the declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

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Desmosomal protein gene mutations in patients with idiopathic dilated cardiomyopathy undergoing cardiac transplantation: a clinicopathological study

Pablo Garcia-Pavia, Petros Syrris, Clara Salas, et al.

Heart 2011 97: 1744-1752 originally published online August 22, 2011
doi: 10.1136/hrt.2011.227967

Notes