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Familial Dilated Cardiomyopathy: Evidence for Genetic and Phenotypic Heterogeneity

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OBJECTIVES This study was performed to evaluate the characteristics, mode of inheritance and etiology of familial dilated cardiomyopathy (FDC).

BACKGROUND A genetic form of disease transmission has been identified in a relevant proportion of patients with dilated cardiomyopathy (DCM). Variable clinical characteristics and patterns of inheritance, and an increased frequency of cardiac antibodies have been reported. An analysis of FDC may improve the understanding of the disease and the management of patients.

METHODS Of 350 consecutive patients with idiopathic DCM, 281 relatives from 60 families were examined. Family studies included clinical examination, electrocardiography, echocardiography and blood sampling. Of the 60 DCM index patients examined, 39 were attributable to FDC and 21 were due to sporadic DCM. Clinical features, histology, mode of inheritance and autoimmune serology were examined, molecular genetic studies were undertaken and the difference between familial and sporadic forms was analyzed.

RESULTS Only a younger age ($p = 0.0005$) and a higher ejection fraction ($p = 0.03$) could clinically distinguish FDC patients from those with sporadic DCM. However, a number of distinct subtypes of FDC were identified: 1) autosomal dominant, the most frequent form (56%); 2) autosomal recessive (16%), characterized by worse prognosis; 3) X-linked FDC (10%), with different mutations of the dystrophin gene; 4) a novel form of autosomal dominant DCM with subclinical skeletal muscle disease (7.7%); 5) FDC with conduction defects (2.6%), and 6) rare unclassifiable forms (7.7%). The forms with skeletal muscle involvement were characterized by a restrictive filling pattern; the forms with isolated cardiomyopathy had an increased frequency of organ-specific cardiac autoantibodies. Histologic signs of myocarditis were frequent and nonspecific.

CONCLUSIONS Familial dilated cardiomyopathy is frequent, cannot be predicted on a clinical or morphologic basis and requires family screening for identification. The phenotypic heterogeneity, different patterns of transmission, different frequencies of cardiac autoantibodies and the initial molecular genetic data indicate that multiple genes and pathogenetic mechanisms can lead to FDC. (J Am Coll Cardiol 1999;34:181–90) © 1999 by the American College of Cardiology

Dilated cardiomyopathy (DCM) is a disease of the myocardium associated with dilation and impaired contraction of the left or both ventricles (1). Dilated cardiomyopathy represents a major cause of morbidity and mortality among cardiovascular diseases, and is a leading indication for heart transplantation. Viral persistence, the presence of an autoimmune response against myocardial epitopes and genetic factors are believed to play a major pathogenic role (1).

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Abbreviations and Acronyms

AD = autosomal dominant
AR = autosomal recessive
CK = creatine kinase
DCM = dilated cardiomyopathy
FDC = familial dilated cardiomyopathy
MDDC = autosomal dominant dilated cardiomyopathy with subclinical muscle involvement
XLDC = X-linked dilated cardiomyopathy

Familial dilated cardiomyopathy (FDC) was very rarely reported in the past (2). Nevertheless, studies based on a more careful evaluation of disease inheritance (3,4) and, more recently, on a prospective systematic family screening, revealed a high frequency of genetic transmission of the disease, ranging from 20% to 35% in different populations (5–7). However, the true frequency is probably still underestimated due to the absence of early markers of the disease and reduced penetrance. Furthermore, affected individuals can be missed in small families and there is an absence of reliable markers to discriminate between the familial and the sporadic forms of DCM (5–7).

The occurrence of genetic transmission indicates the existence of a defective gene (or genes). Familial dilated cardiomyopathy is probably a complex trait with a major gene causing the disease and other factors altering its expressivity, such as modifier genes and environmental factors. Among potential modifier genes (or susceptibility factors), an abnormal immune response has long been suspected. Circulating cardiac-specific autoantibodies were detected in 20% of first-degree relatives of DCM patients and in 58% of the pedigrees studied, including both FDC and sporadic cases (8).

To understand the phenotype and the molecular basis of FDC, a prospective survey was undertaken in 1991 in a series of 350 consecutive patients diagnosed with DCM. In particular, the purpose of this study was to investigate the clinical and instrumental features of the disease, the modes of inheritance, the characteristics of the immune response and the differences between the sporadic and familial forms to establish a basis for the detection of the specific genetic defects.

METHODS

Patient population. From 1991 to July 1997, 511 patients with primary cardiomyopathies were studied in our Institution, as approved by the institutional review committee and after obtaining informed consent. Of them, 350 (68%) were classified as idiopathic DCM. All DCM patients were evaluated by clinical examination, electrocardiography (standard electrocardiography, Holter monitoring and signal-averaged electrocardiography) and echocardiography (M mode, cross-sectional and Doppler). The data were interpreted by two independent observers. Normal values for echocardiographic measurements were determined according to standard protocols (9). Additional investigations routinely performed on index patients included standard laboratory examinations (including serum creatine kinase [CK]), chest X ray, exercise test, radionuclide angiography and an extensive invasive evaluation, including ventriculography, coronary angiography and endomyocardial biopsy.

The assessment of DCM was based on strict diagnostic criteria (1,10) requiring the presence of depressed left ventricular systolic function (fractional shortening $<25\%$ and/or ejection fraction $<45\%$), associated with left ventricular dilation (left ventricular end-diastolic diameter $>117\%$ of the predicted value corrected for the age and body surface area [11]), in the absence of any known cause of heart disease. Exclusion criteria were: moderate to severe arterial hypertension, coronary artery disease, arrhythmogenic right ventricular dysplasia, excessive alcohol consumption, high rate supraventricular arrhythmia, significant valvular heart disease, systemic diseases, pericardial diseases, congenital heart disease and cor pulmonale. Histologic signs of myocarditis were not considered exclusion criteria.

Family studies. For each DCM index patient, a detailed two- to six-generation pedigree was constructed. Since the aim of this study was an extensive analysis of the phenotype and molecular basis of FDC, the selection of the families was based on their availability for family screening. A noninvasive familial screening was offered, regardless of family history, and then performed in all available relatives by means of clinical examination, electrocardiography, echocardiography and, in selected cases, signal-averaged electrocardiography. Relatives showing signs of myocardial disease underwent full invasive evaluation as described for the index patients (see preceding section), with the exclusion of six subjects under 20 years of age and seven adults unable to undergo invasive studies. In these cases, noninvasive investigation (10,12) and a review of previous hospital records completed the evaluation. For the deceased relatives, hospital records were examined when available, family physicians were interviewed and multiple informants among close relatives were consulted for accuracy of diagnosis. Criterion for the diagnosis of FDC was the presence of at least two family members with documented DCM. After the identification of genetic transmission, family members were invited to participate in a follow-up program with periodic examinations to evaluate the evolution of the clinical status in both affected and unaffected individuals and to avoid the risk of misdiagnosis. Two hundred clinical and instrumental parameters were recorded in a database for statistical analysis.

Histologic studies. Endomyocardial biopsy from the right or the left ventricle was performed in 83 affected individuals during diagnostic heart catheterization. The number of biopsies ranged from four to five specimens in each patient. The biopsies were processed and analyzed as reported in detail elsewhere (13). Active myocarditis was assessed ac-

cording to the Dallas criteria (14). Morphometric analysis was performed in 44 cases and assessed as previously described (13), with at least three samples from each patient being examined to obtain a mean value for each parameter.

To examine skeletal muscle for the presence of pathologic changes, a needle biopsy of the quadriceps was obtained from 14 patients available for this study, after informed consent. Signs of skeletal myopathy or an abnormal serum CK were not required. The tissue samples were prepared and stained according to standard histologic procedures. Immunohistochemical studies on endomyocardial and skeletal muscle samples were performed as previously described (15).

Genetic, immunologic and immunogenetic studies. Once the diagnosis was established, blood, sera, deoxyribonucleic acid and lymphoblastoid cell lines (deriving from immortalization of B lymphocytes with Epstein-Barr virus) were collected and stored. Samples were obtained from all index patients and from each available family member. Methods for gene mapping were based on the analysis of microsatellite polymorphic markers and linkage analysis as described in detail elsewhere (16). Methods for the detection of mutations in the dystrophin gene were based on multiplex polymerase chain reaction, single-strand conformation polymorphism, restriction enzyme analysis and sequence analysis, as previously reported (17). Serum samples of 73 relatives (affected and unaffected) from families with FDC were tested for the presence of organ-specific cardiac autoantibodies using standard indirect immunofluorescence, as described (8,18). Human leukocyte antigen (HLA) typing was performed in 51 subjects (affected and unaffected relatives from families with FDC) on peripheral blood lymphocytes isolated from heparinized whole blood by separation on histopaque ficoll density gradient. HLA-DR typing was performed with the standard lymphocytotoxicity assay.

Statistical analysis. The method for the identification of differences between the familial and the sporadic forms of DCM, and among the different subtypes of FDC, was based on the uni- and multivariate analysis of 200 clinical, instrumental and morphologic parameters. Mean and median values or percentages, where appropriate, were calculated for all clinical and instrumental variables. The Univariate Odds Ratio was computed via a logit model. The test for significance of the difference in the mean values among the groups was based on the Wilcoxon test. For the categorical variables, the chi-square test (continuity-adjusted if appropriate) was computed. Multivariate analysis performed to develop a predictive model was based on all variables that were shown to be significant in the univariate analysis at a threshold of 0.25 (19). Variables were selected for the resulting logit model using a stepwise selection procedure, based on the Akaike Information Criterion using a threshold of 0.05. In this study, the criteria for the family selection were not designed for an estimation of the prevalence of FDC.

Table 1. Study Population

Patients with dilated cardiomyopathy (n)	350
Families clinically investigated (n)	60
Subjects examined (n)	281
Affected (n)	96
Unaffected (n)	170
Unknown status (n)	15
Families with familial dilated cardiomyopathy (n)	39
Families with sporadic dilated cardiomyopathy (n)	21
Relatives studied in familial dilated cardiomyopathy (%)	
I degree	20
II degree	30
III degree	50
Relatives studied in sporadic dilated cardiomyopathy (%)	
I degree	77
II degree	19
III degree	4

RESULTS

Family studies. Sixty families of DCM patients were extensively investigated irrespective of family history. An overall number of 281 family members underwent family screening (Table 1). Relatives were assessed as affected when they met the full criteria for DCM, as unaffected when they had a normal heart or other causes of heart disease, or as unknown in cases with minor cardiac abnormalities. According to these criteria, 96 of the relatives were affected, 144 were unaffected and 15 were considered as unknown. The clinical features of the relatives with unknown status were the following: left ventricular dilation (eight cases), left bundle branch block (four), arterial hypertension (six), complex ventricular arrhythmia (two), segmental hypokinesia (one) and skeletal myopathy (three). Twenty-six spouses of affected family members were studied for the analysis of the genetic transmission of the disease, but not included in the statistical analysis of the phenotype.

As defined, FDC was present in 39 families, whereas in 21 families the condition was confirmed to be sporadic. At the time of the initial evaluation, familial transmission of the disease had not been identified in three out of 24 cases with no family history, leading to a misclassification error of 12.5% due to the absence of clinical family screening data.

Analysis of the differences between FDC and sporadic DCM. In the comparison between FDC and sporadic DCM only the probands were considered (39 and 21, respectively) to avoid ascertainment bias, that is, early disease identified by family screening. Of the 200 parameters analyzed, only three showed a significant difference between the two groups (Table 2); FDC patients were characterized by an increased myocardial fibrosis using the morphometric analysis, a younger age and a trend toward a higher left ventricular ejection fraction compared with

Table 2. Analysis of the Difference Between Sporadic and Familial Dilated Cardiomyopathy at Uni- and Multivariate Analysis

	Sporadic DCM (21 Cases)	FDC (39 Cases)	p
Collagen volume fraction (%)	21.5 (10.5–28)	37 (20.2–45.7)	0.039
Age (yr)	52 (46–55)	37 (26–45)	0.0005*
LVEF (%)	27 (19–32)	30 (24–39)	0.0306*

*p values at multivariate analysis. Data are expressed as median (first and third quartile).
DCM = dilated cardiomyopathy; FDC = familial dilated cardiomyopathy; LVEF = left ventricular ejection fraction.

sporadic DCM. However, only the last two parameters were independent in the multivariate analysis. Moreover, no significant differences were found between the healthy relatives of patients with FDC (96 subjects) and those with sporadic DCM (48 subjects). In this analysis, 15 members of FDC families with unknown status were not included.

Characterization and classification of FDC. The analysis of the familial forms revealed broad genetic heterogeneity, based on different clinical features, different patterns of genetic transmission and, when available, molecular genetic data. The classification and the features of the FDC types are described in detail in Table 3, an example of pedigree for each subtype of FDC is illustrated in Figure 1 and an outline of the most important characteristics of each group is given below.

1. Autosomal dominant (AD) pure FDC was identified in the majority of families (56%). This form was characterized by variable and age-related penetrance (<20 years: 10%, 20 to 30: 34%; 30 to 40: 60%; >40: 90%), with onset generally in the fourth decade of life. The clinical and histologic examinations of skeletal muscle appeared to be normal. Mildly dilated forms (20,21) were frequent (42%), as were segmental wall motion abnormalities in the early phases of the disease. In one case, an idiopathic left ventricular aneurysm was observed (22). In another family, evidence of histologic myocarditis was found in one affected individual (Fig. 2). Organ-specific cardiac autoantibodies were very frequent in this group (39% of cases were positives), in both affected (72%) and unaffected (27%) family members. In one large family (Fig. 1, AD-FDC1) and in two other small families, the disease locus was mapped to chromosome 9 (16).
2. Autosomal recessive (AR) FDC was present in 16% of our families (Fig. 1, AR-FDC3). The patient age was significantly younger compared with the AD form ($p = 0.05$) and the course of the disease showed a rapid progression to death or transplant, in less than one year in 50% of cases, all of which were under 21 years of age ($p < 0.05$). Two patients belonging to two different families showed evidence of myocarditis.
3. Autosomal dominant conduction disease with DCM was rare in our patient population (Fig. 1, family CDDC1). Typically, as described by Graber et al. (23), in the two affected family members the onset of the disease was characterized by atrioventricular blocks (second and third degree) and intraventricular conduction delays (left bundle branch block) requiring a permanent pacing, and supraventricular (atrial flutter) as well as ventricular arrhythmia. In the early phase of disease, the patients showed only mild ventricular dysfunction. However, progressive worsening of heart failure rapidly followed, requiring heart transplantation in one case.
4. Four families (10%) were included in the group with X-linked DCM (Table 3, XLDC). They were characterized by X-linked inheritance with no male to male transmission. In the affected subjects (all male), the onset appeared later than previously described, with symptoms of severe progressive heart failure, typically in the absence of overt clinical signs of skeletal myopathy. Mildly dilated forms were also observed in these patients. While a characteristic increase of CK (isoform MM) levels was observed in this form of the disease, CK levels were found to be normal in one case. All but one of the obligate carriers were normal (Fig. 1). A mutation of the dystrophin gene was demonstrated in the three families available for molecular diagnosis. In the first family, a point mutation at the 5′ end of the dystrophin gene was found, consisting of a G to T transversion that altered the consensus sequence (AG) at the first muscle exon–intron junction (17). In the second family (Fig. 1, family XLDC2), multiplex polymerase chain reaction analysis showed a deletion occurring in exon 48–49 (24). In the third family, a deletion of the muscle-promoter region and the first muscle exon had been previously identified (25). Even in the absence of muscle weakness, skeletal muscle biopsies showed myopathic changes, such as variability in fiber size, rare splitting and an increase in the number of internal nuclei. The endomyocardial biopsy was characterized by fibrosis and, accordingly, morphometric analysis showed a trend toward an increased collagen volume fraction and reduced myocyte, nuclear and myofibrillar areas (Table 3). Immunocytochemical studies with antidystrophin antibodies were diagnostic, showing that in the skeletal muscle the protein was reduced in quantity, even if normally localized, whereas dystrophin was undetectable in the cardiac muscle (17,24,26).
5. The fifth subtype was AD DCM with subclinical skeletal muscle disease. Of FDC subjects, 7.7% presented a marked variability of expression (Table 3, MDDC). The phenotype was characterized by no clinical difference between affected female and male subjects, variable degree of skeletal muscle involvement, presence of conduction defects, frequent ventricular arrhythmia and variable levels of the serum CK-MM, from overtly abnormal to normal values even in the same subjects, at different stages of the disease. In two of the seven affected,

Table 3. Summary of Clinical, Immunologic, Morphometric and Molecular Genetic Findings in Familial Dilated Cardiomyopathy (FDC)

FDC Type	AD FDC	AR FDC	XLDC	MDDC	Unclassifiable		CDDC
					AD HDC	AR RPDC	
Clinical features							
No. of kindreds (%)	22 (56)	6 (16)	4 (10)	3 (7.7)	2 (5.1)	1 (2.6)	1 (2.6)
Studied subjects (n)	132	16	6	22	8	2	2
Affected	53	10	4	7	5	2	2
Unknown status	10	0	0	5	0	0	0
Age at diagnosis, yr (range)	43* (4–82)	21* (7–53)	29 (19–39)	34 (16–46)	34 (18–46)		42 (40–43)
Disease duration, mo (range)	26 (0–120)	31 (2–120)	6 (1–13)	21 (3–108)	22 (2–64)		24 (12–36)
Gender (male:female)	3:2	4:1	4:0	6:1	1:1		2:0
LVEDD (cm)	6.6 ± 1.5	6.3 ± 0.9	6.7 ± 1.8	6.5 ± 1.4	5.6 ± 1.0		6.0
LVEF (cm)	34 ± 11	31 ± 12	32 ± 24	28 ± 12	39 ± 16		(FS 23%)
Mildly dilated, n (%)	22 (42)	2 (20)	2 (50)	4 (57)	4 (57)		0
LBBB, n (%)	8 (16)	2 (20)	0	6 (86)	0	2/2	2/2
Permanent pacing	0	0	0	1	0	2/2	2/2
Ventricular tachycardia, nonsustained and sustained (%)	36 (72)	3 (43)	3 (75)	5 (83)	0	2/2	2/2
CK-MM, mean times normal (range)	NV	NV	3.7 (NV–10×)	2.5 (NV–3.6×)	NV	NV	NV
Cardiac autoantibodies							
No. of relatives tested (% positive)	21 (39)	6 (33)	3 (0)	11 (9)	—	1 (0)	—
No. of affected tested (% positive)	18 (72)	4 (50)	2 (0)	1 (14)		1 (0)	
Histology and morphometry							
Myocarditis (14) (No. of cases)	1	2	0	2	2	0	0
Morphometry (No. of study cases)	26	6	4	5	2	1	0
Myocellular area (μm^2)	1.9 ± 0.8	2.2 ± 0.6	1.6 ± 0.7	1.7 ± 0.6	1.8 ± 0.4	2.0 ± 0.8	—
Nuclear area (μm^2)	0.1 ± 0.1	0.2 ± 0.04	0.1 ± 0.05	0.1 ± 0.05	0.1 ± 0.1	0.2 ± 0.01	—
Myofibrillar area (μm^2)	1.1 ± 0.6	0.9 ± 0.5	1.0 ± 0.52	1.5 ± 0.8	1.0 ± 0.3	0.8 ± 0.5	—
Collagen volume fraction (%)	13.8 ± 9.7	6.6 ± 5.4	17.3 ± 15.0	13.8 ± 11.6	7.4 ± 3.5	0	—
Adipose tissue (%)	6.5	0	0	0	0	0	—
Skeletal muscle biopsy (no. of cases)	Normal (6)	—	Dystrophic changes (3)	Dystrophic changes (5)	—	—	—
Molecular genetic data							
Chromosome	9q13-q22 (16)	Unknown	Xq21	Unknown	Unknown	Unknown	Unknown
Gene	Unknown	Unknown	Dystrophin	Cytoskeletal proteins?	Unknown	mtDNA? Autosomal?	Unknown
Mutation			1. Del MP-ME1 (25) 2. Del E 48-49 (24) 3. II G→ T splice donor site (17)				
Follow-up							
Major events at 1 yr (%)	0†	5 (50)†	0	2 (28)	1 (20)	0	0

*Age difference $p = 0.05$. †Age difference $p < 0.05$. Disease duration = from first symptom to diagnosis. Major events = deaths and heart transplants. Mildly dilated = left ventricular end-diastolic diameter corrected for body surface area $<3.2 + 15\% = 3.68$ cm/m^2 (20,21). The morphometric data refer to the mean value derived from at least three different samples for each patient.

AD = autosomal dominant; AR = autosomal recessive; CDDC = conduction defect with dilated cardiomyopathy; CK-MM = creatine kinase, isoform MM; Del = deletion; FS = fractional shortening; HDC = familial dilated cardiomyopathy with apical hypertrophy; I1 = first intron; LBBB = left bundle branch block; LVEDD = left ventricular end-diastolic diameter; LVEF = angiographic left ventricular ejection fraction; MDDC = autosomal dominant dilated cardiomyopathy with skeletal muscle involvement; ME1 = first muscle exon; MP = muscle promoter; mtDNA = mitochondrial deoxyribonucleic acid; NV = normal values; RPDC = familial dilated cardiomyopathy associated with retinitis pigmentosa; XLDC = X-linked dilated cardiomyopathy.

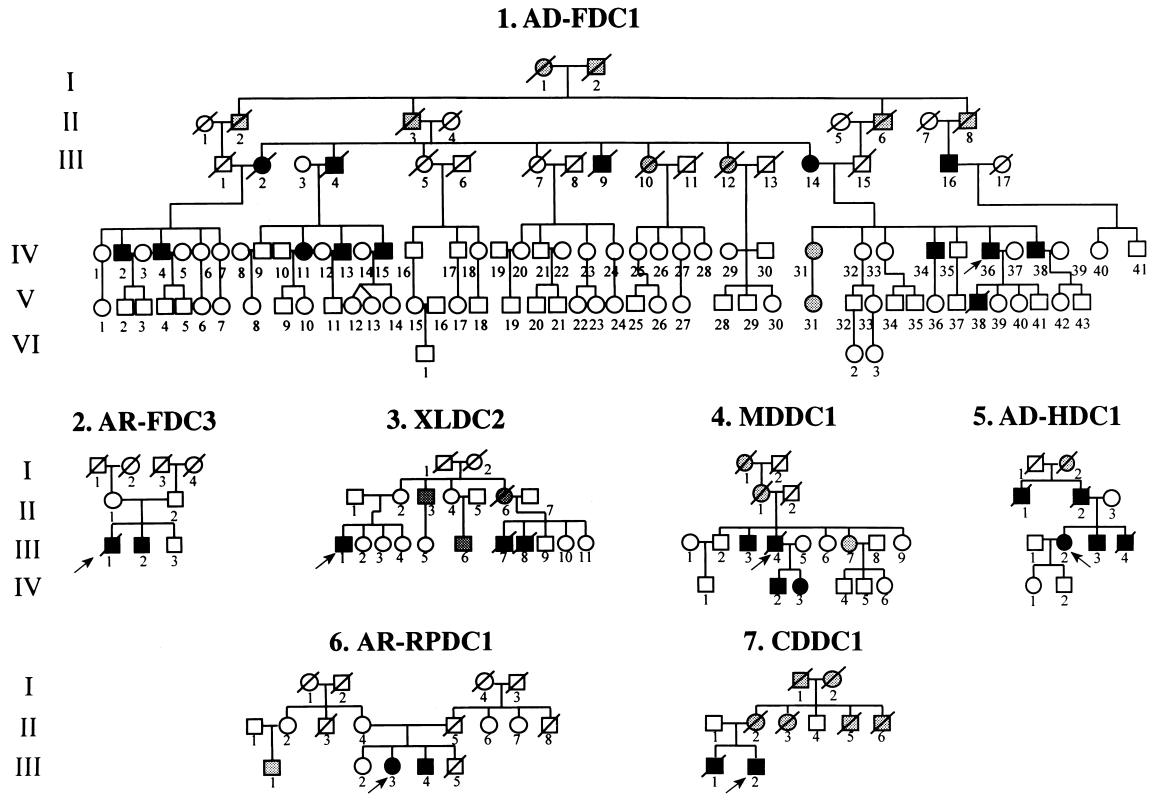

Figure 1. Pedigrees of families with different forms of familial dilated cardiomyopathy (FDC). **1**. AD-FDC1: the key members of this family after the last follow-up screening (1997), consistent with autosomal dominant inheritance (male to male transmission) and age-related penetrance (almost complete absence of affected in the last generation). **2**. AR-FDC3: autosomal recessive FDC, in which the affected were respectively 12 and 16 years old at diagnosis. **3**. XLDC2: FDC with X-linked transmission, in this family due to a deletion of exons 48–49 (24). **4**. MDDC1: autosomal dominant transmission and subclinical skeletal muscle involvement. **5**. AD-HDC1: a form of unclassifiable FDC with autosomal dominant transmission and a phenotype characterized by mild dilation, systolic dysfunction and apical hypertrophy. **6**. AR-RPDC1: another unclassifiable FDC with autosomal recessive transmission associated with retinitis pigmentosa and hearing loss. Finally, CDDC1: autosomal dominant family with conduction defects and subsequent development of severe ventricular dilation and dysfunction. Individuals are indicated by generation and pedigree number. Affected status is indicated by **filled symbols**, unaffected status by **clear symbols** and unknown status (individuals with equivocal or suspected dilated cardiomyopathy) by **gray symbols**. The probands are indicated by **arrows**.

the rapid and progressive worsening of heart failure led to death within 1 year from the diagnosis. Mildly dilated forms and segmental hypokinesia were observed in this group. Histologic signs of myocarditis were found in two cases (Fig. 1, MDDC1 family). The skeletal muscle biopsy showed dystrophic changes. As expected, dystrophin was normal in all.

6. Unclassifiable FDC was a heterogeneous group including three families (7.7 %) with peculiar phenotypes. The first group included two families characterized by hypokinetic left ventricle with localized apical hypertrophy of the left or both ventricles (Fig. 1, AD-HDC1 family, and Fig. 3), named AD DCM with hypertrophy (Table 3). Histologic signs of myocarditis were also observed in this group. Autosomal recessive transmission, retinitis pigmentosa and deafness characterized the second type (Fig. 1, AR-RPDC1 family). The patients presented a stable course of the disease after a follow-up to 7.5 years. The left ventricle was only mildly dilated. Serum CK, lactate and karyotype were normal; mitochondrial deoxyribonucleic acid analysis revealed absence of macrodeletions and of the most common point mutations.

Search for predictive parameters within the familial forms. To identify specific features, and to have a statistically representative sample, the different forms of FDC were clustered into two groups: patients with isolated myocardial disease (including 63 affected members of 28 families with AD or AR FDC) and patients with skeletal muscle involvement (including 11 affected members of seven families with X-linked dilated cardiomyopathy [XLDC] and AD dilated cardiomyopathy with subclinical muscle involvement [MDDC]). Families with unclassifiable forms were not included in this analysis. The results of the uni- and multivariate analysis are reported in Table 4. Familial dilated cardiomyopathy forms with skeletal muscle involvement were characterized by clinical and echocardiographic restrictive filling patterns and by a significant increase of

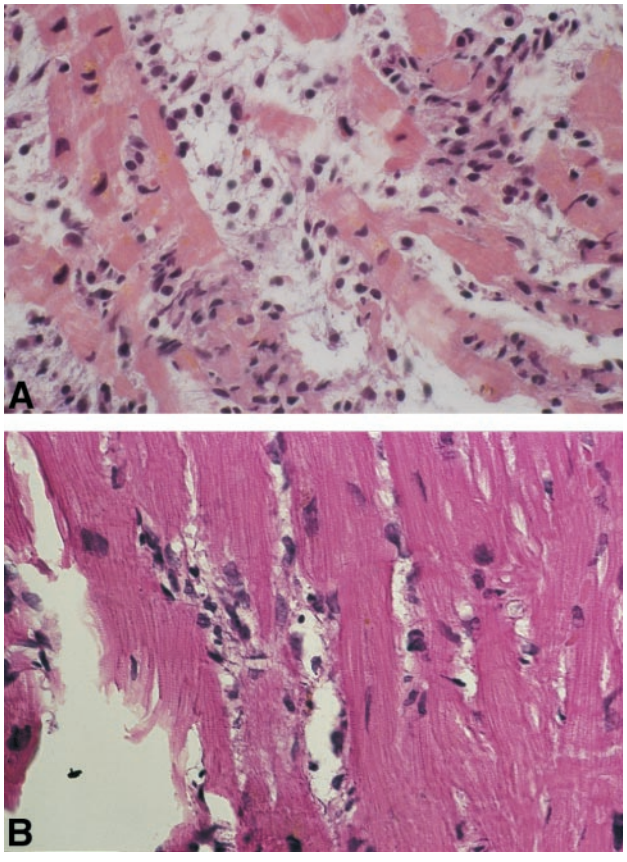

Figure 2. Samples from the proband of a family with autosomal dominant familial dilated cardiomyopathy showing myocarditis at the histologic examination of the endomyocardial biopsy. **(A)** Acute active myocarditis, with massive infiltration of lymphocytic, monocytic and plasma cells, necrosis of adjacent myocardial fibers, interstitial edema and disarrangement of the muscle bundles (hematoxylin–eosin, ×120, reduced by 54%). **(B)** Healing myocarditis, with interstitial edema and a focal inflammatory infiltration with myocyte necrosis (hematoxylin–eosin, ×180, reduced by 54%).

serum CK-MM. On the other hand, pure FDC was characterized by the presence of cardiac autoantibodies, which was the only independent predictor. Human leukocyte DR4 antigen did not correlate with the presence of autoantibodies in these patients, and was not associated with the disease status in the overall FDC population.

In the familial forms, the affected status was significantly associated with the male gender (2:1 vs. 1:1, $p = 0.046$), also after exclusion of XLDC, and with the presence of organ-specific cardiac autoantibodies (16% vs. 2%, $p = 0.003$), which did not correlate with the human leukocyte DR4 antigen.

Symptomatic ($n = 29$) versus asymptomatic ($n = 44$) affected family members were characterized, as expected, by all parameters indicative of left ventricular dysfunction and dilation. However, no independent predictors were observed at the multivariate analysis.

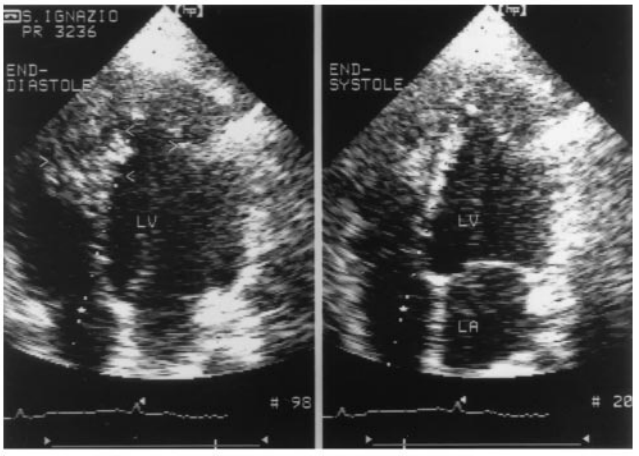

Figure 3. Echocardiographic features of autosomal dominant hypertrophic–hypokinetic cardiomyopathy (AD-HDC). Apical four-chamber view of individual III-3 of family AD-HDC1 (see Fig. 1). **Left frame:** end-diastole; **right frame:** end-systole. The left ventricle (LV) is only mildly dilated (end-diastolic volume 112 ml); a severe apical hypertrophy is present **(arrows)**. The left ventricle is diffusely hypokinetic, with moderate systolic dysfunction (ejection fraction 44%). LA = left atrium.

DISCUSSION

Identification of FDC. In this survey, the occurrence of an inherited trait was detected in 39 out of 60 families of patients with idiopathic DCM. The extensive analysis of clinical and morphologic features of the 60 index patients shows that there were no reliable clinical or morphologic parameters able to predict the familial form. In this series, only a more advanced age and a worse ventricular function characterized the sporadic versus the familial cases, suggesting different stages of the disease, rather than different diseases. Likewise, no differences were found between healthy relatives of sporadic and familial DCM patients. The lack of differential features was probably due to the etiologic heterogeneity of both groups.

These data, as well as the potential risk of misclassification error (12.5% in this population) without a family screening, stress the need for clinical examination of at least the first-degree relatives of DCM patients, regardless of their family history. The disease can be asymptomatic or clinically not evident due to the reduced and age-related penetrance. The family screening can detect initial cardiac abnormalities in individuals of unknown status which may represent early manifestations of the disease. These abnormalities, particularly frequent in the AD form (20% of the patients' relatives), have been shown to progress to overt DCM in 27% of subjects (27).

Concerning the etiology of sporadic DCM, this could be due to environmental factors such as viral infections, toxic agents such as alcohol, autoimmunity (8) or multifactorial mechanisms. However, a portion of the apparently sporadic cases could also be caused by gene defects. The absence of observed genetic transmission in these cases may be due to

Table 4. Uni- and Multivariate Analysis of the Difference Between Familial Dilated Cardiomyopathy (FDC) With and Without Skeletal Muscle Involvement

	"Pure" FDC (63 Patients)	FDC With Myopathy (11 Patients)	p
Third heart sound (%)	27	64	0.020
Restrictive pattern (%)	6	40	0.054
LVEDP (mm Hg)*	12 (8–20)	21 (15–26)	0.022
Serum CK (U/liter)*	52 (37–89)	242 (83–423)	0.008
Organ-specific autoantibodies (%)	68	9	0.0196†

*Data are expressed as median (first and third quartile). †p value at multivariate analysis.
Restrictive pattern = on echocardiography. CK = creatine kinase; LVEDP = left ventricular end-diastolic pressure.

de novo mutations such as those reported in hypertrophic cardiomyopathy (28), or reduced penetrance in the carriers masking their affected status or insufficient family data.

Clinical and molecular characterization of FDC. In this study, the analysis of the phenotype, the genetic transmission and, when available, the molecular genetic data revealed distinctive features among the different forms of FDC, leading to a new classification of the familial forms in our patient population. Our findings support the hypothesis of genetic heterogeneity in FDC, which means that different genes can cause the same syndrome.

In our population, the most common type of FDC was the AD form with isolated myocardial involvement (56% of our families). Autosomal dominant FDC was characterized by mildly dilated forms in 42% of cases and by the presence of cardiac autoantibodies in 72% of the affected individuals, compared with 15% of sporadic cases and 3.5% of control subjects (8). The identification of a high frequency of cardiac autoantibodies in the forms with pure heart muscle disease suggests an activation of an immune response, which was independent from human leukocyte DR4 antigen. There is no conclusive evidence that these antibodies are directly pathogenetic (29), but they may represent reliable markers of autoimmunity and may predict early disease among relatives at risk of developing DCM (8).

Molecular genetic studies based on linkage analysis have allowed the identification of four loci for AD FDC: on chromosome 9 in three of our families (16), on chromosome 1q32 in another kindred (30), on chromosome 10q21-23 in a family characterized by high penetrance and mitral prolapse (31) and, very recently, on chromosome 2q31 in one family (55).

The AR form of the disease was less frequent (16%). In these families, both parents of the index patient were found to be unaffected. Autosomal recessive FDC was characterized by a significantly younger age and a worse prognosis compared with the dominant form, suggesting two distinct etiologies.

Dilated cardiomyopathy with conduction defects was rare in our series (2.6%). A peculiarity of this form was the onset with severe conduction defect and only minor ventricular dysfunction, subsequently followed by progressive heart failure, as previously described (23). In this form, two disease loci have been reported, one on chromosome 1p1-q1 (32) and the other on 3p22-p25 (33); the disease genes mapping to these loci are still unknown.

Also rare in our study population were families with unclassifiable FDC (7.7%). Two of these families showed a peculiar phenotype characterized by apical hypertrophy and AD transmission (Fig. 2). The third family was characterized by a complex phenotype, with hearing loss, retinitis pigmentosa and apparently AR transmission. This phenotype, also observed in other patient populations (7), has been reported in rare mitochondrial disorders (34,35), as well as in AR disorders such as Alstrom syndrome (OMIM [Online Menolelian Inheritance in Man] #203800), Usher syndrome (OMIM #276900) and Leber I syndrome (OMIM #204000).

Most of the knowledge of the molecular basis of DCM and of the genotype/phenotype correlation is due to the identification of the gene causing XLDC (25,36), the dystrophin gene. This gene encodes a large cytoskeletal protein which plays a critical role in membrane stability, force transduction and organization of the membrane in skeletal and cardiac myocytes (37). X-linked dilated cardiomyopathy was previously described in male teenagers, as a rapidly progressive congestive heart failure, without clinical signs of skeletal myopathy but with elevated serum CK-MM (38). However, in our patients we observed an older age, a less severe prognosis and, in one case, a normal serum CK, indicating variable expressivity of this form of the disease. These findings underline the need for a systematic and accurate skeletal muscle examination in FDC, even in the absence of overt signs of myopathy. In XLDC, the skeletal muscle biopsy is diagnostic and the definition of the molecular defect is possible.

Molecular genetic analysis of the dystrophin gene demonstrated three different mutations in our families. Two of them were in the 5′ end of the gene (17,25), whereas the third was found in exon 48 (24), a region of the dystrophin gene found to be mutated in Becker muscular dystrophy with severe myocardial dysfunction (39). Interestingly, in the first two families, expression studies showed that dystrophin was absent in the heart and partially replaced by the compensatory production of other isoforms (brain and Purkinje) in the skeletal muscles (17,26). Recently, muta-

tions of other regions of the gene have been implicated in XLDC, such as a point mutation in exon 9 (40), a duplication mutation involving exons 2–7 (41) and rearrangements of Alu-like sequences in intron 11 (42). These regions are probably critical for the expression and function of dystrophin in the heart.

The AD form with skeletal muscle involvement (MDDC) is a novel entity, characterized by a highly variable expressivity. A common feature of MDDC and XLDC was a restrictive filling pattern. The gene causing MDDC is unknown, but similar phenotypes were recently described in families with limb girdle muscular dystrophy associated with severe DCM. Two disease loci have been mapped, one to chromosome 1 (43) and the other to chromosome 6 (44). By analogy with dystrophin, other cytoskeletal proteins appear to be potential candidates for causing DCM. A deficiency of alpha sarcoglycan (adhalin) was observed in a patient with muscle dystrophy and DCM (45). The absence of transcription of metavinculin (the cardiac isoform of vinculin) was reported in another FDC patient (46). Furthermore, delta-sarcoglycan has been recently identified as the disease gene of the Syrian hamster (BIO14.6) cardiomyopathy, and mutations in another cytoskeletal gene, MLP, produce cardiomyopathy in mice (47).

Recently, the analysis of candidate genes has allowed the detection of missense mutations in the cardiac actin gene in two small families with DCM (48). Cardiac actin has been traditionally considered a sarcomeric gene. However, in the two families described, the mutations occur in exons 5 (Arg312His) and 6 (Glu361Gly), leading to an altered interaction with the Z band and the intercalated disks. This supports the hypothesis of a critical role of the cytoskeleton and related structures for the integrity of myocardial function, and the concept of a defect of force transmission in DCM, in contrast to the defect of force generation due to mutations of sarcomeric proteins in hypertrophic cardiomyopathy.

In this study, myocarditis, as defined by the Dallas criteria (14), as well as mildly dilated cardiomyopathy and segmental wall motion abnormalities were detected in different subgroups. The frequent finding of myocarditis is not surprising, since histologic inflammation could represent the acute stage of an autoimmune activation, particularly in association with cardiac-specific autoantibodies (1), or conversely, an unspecific response to tissue injury mediated by other noxae.

The molecular mechanisms causing the disease and the genotype/phenotype correlations need to be fully defined. Most of the genes causing FDC are still unknown, but it is clear that their identification will have a considerable impact in the prevention of DCM and treatment of DCM patients. Besides the major disease genes, the variable penetrance and expressivity of the disorder, and the different gender susceptibility, suggest the existence of modifier genes that could affect the manifestation and severity of the disease. For instance, genes of the renin–angiotensin system seem to influence the severity of heart failure in ischemic and idiopathic DCM (49), and are believed to influence the development of hypertrophy in hypertrophic cardiomyopathy (50,51). Moreover, a complex genetic basis could account for the autoimmune subset of FDC, in keeping with the polygenic nature of other autoimmune conditions, for example, type 1 insulin-dependent diabetes mellitus (52). Finally, the increased male/female ratio observed in forms with evidence of autosomal transmission suggests the existence of susceptibility factors associated with gender. The elucidation of the mechanisms of these susceptibility or modifier genes could provide other tools for the management of DCM.

Conclusions. The identification of a genetic background in a large proportion of patients and the initial results of clinical and molecular genetic studies showing genetic heterogeneity represent important progress in the understanding of DCM and have relevant implications. The management and the study of FDC require more sensitive and specific diagnostic criteria; this need prompted the European Collaboration on FDC to develop new guidelines for family studies (53), which could represent the basis for a common approach to the genetics of DCM, both in the research and in the clinical context.

Several aspects still need to be fully elucidated, including the natural history of FDC. In this study, minor cardiac abnormalities were frequently seen in patients' relatives which could be early signs of disease (27). Furthermore, previous studies reported a severe prognosis for FDC (54), but our data suggest that different subtypes can have different evolutions. Follow-up studies are needed to answer these questions.

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