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Research Article

Dilated cardiomyopathy (DCM) is the most common form of primary myocardial disorder, accounting for 60% of all cardiomyopathies. In 20-30% of cases, familial inheritance can be demonstrated; an autosomal dominant transmission is the usual type of inheritance pattern identified. Previously, genetic heterogeneity was demonstrated in familial autosomal dominant dilated cardiomyopathy (FDCM). Gene localization to chromosome 1 (1p1-1q1 and 1q32), chromosome 3 (3p25-3p22), and chromosome 9 (9q13-9q22) has recently been identified. We report one family with 26 members (12 affected) with familial autosomal dominant dilated cardiomyopathy in which linkage to chromosome 10 at the 10q21-q23 locus is identified. Using short tandem repeat polymorphism (STR) markers with heterozygosity > 70%, 169 markers (50% of the genome) were used before linkage was found to markers D10S605 and D10S201 with a pairwise LOD score = 3.91, theta = 0, penetrance = 100% for both markers. Linkage to 1p1-1q1, 1q32, 3p25-3p22, and 9q13-9q22 was excluded. We conclude that a new locus for pure autosomal dominant FDCM exists, and that this gene is localized to a 9 cM region of 10q21-10q23. The search for the disease causing gene and the responsible mutation(s) is ongoing.

Gene Mapping of Familial Autosomal Dominant Dilated Cardiomyopathy to Chromosome 10q21-23

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Abstract

Dilated cardiomyopathy (DCM) is the most common form of primary myocardial disorder, accounting for 60% of all cardiomyopathies. In 20–30% of cases, familial inheritance can be demonstrated; an autosomal dominant transmission is the usual type of inheritance pattern identified. Previously, genetic heterogeneity was demonstrated in familial autosomal dominant dilated cardiomyopathy (FDCM). Gene localization to chromosome 1 (1p1-1q1 and 1q32), chromosome 3 (3p25-3p22), and chromosome 9 (9q13-9q22) has recently been identified. We report one family with 26 members (12 affected) with familial autosomal dominant dilated cardiomyopathy in which linkage to chromosome 10 at the 10q21-q23 locus is identified. Using short tandem repeat polymorphism (STR) markers with heterozygosity > 70%, 169 markers (50% of the genome) were used before linkage was found to markers D10S605 and D10S201 with a pairwise LOD score = 3.91, θ = 0, penetrance = 100% for both markers. Linkage to 1p1-1q1, 1q32, 3p25-3p22, and 9q13-9q22 was excluded. We conclude that a new locus for pure autosomal dominant FDCM exists, and that this gene is localized to a 9 cM region of 10q21-10q23. The search for the disease causing gene and the responsible mutation(s) is ongoing. (*J. Clin. Invest.* 1996. 98:1355–1360.) Key words: dilated cardiomyopathy • genetic heterogeneity • linkage

Introduction

The cardiomyopathies, disorders that primarily affect the myocardium (1), are leading causes of morbidity and mortality in children and adults (2, 3). Dilated cardiomyopathy (DCM)[1] is the most common form of cardiomyopathy (4–6), responsible for approximately 60% of cases of cardiomyopathy with an annual incidence estimated to be 5–8 cases per 100,000 population (6, 7). Many causes for DCM have been described (8), but most commonly this disease is considered idiopathic. Classically, patients present with signs and symptoms of congestive heart failure (1, 2, 9), although many patients are asymptomatic (2, 10). The diagnosis is dependent on echocardiographic evidence of ventricular dilatation and dysfunction (usually affecting the left ventricle predominantly) (11, 12), and exclusion of other causes. Premature death, particularly sudden cardiac death from either ventricular arrhythmias or ischemia, is common (13–15). DCM is the leading indication for cardiac transplantation (16), costing more than $200 million annually (17). In approximately 20–30% of cases of DCM, a familial inheritance (18, 19) (familial DCM, FDCM) is observed with autosomal dominant transmission being the most common transmission pattern. The underlying genetic defect(s) is not currently known.

In recent years, molecular genetic approaches to cardiovascular disease have begun to open the doors to understanding the underlying causes of cardiac disease. Recently, genes for autosomal dominant FDCM have been mapped to chromosome 1p1-1q1 in one family (20), 3p25-3p22 (21) in one family, 1q32 in one family (22), and 9q13-9q22 in three families (23), and thus genetic heterogeneity has been demonstrated (24). None of the genes or protein products responsible for FDCM have been identified to date, however. In other families with similar DCM phenotypes but a different genetic transmission pattern, two X-linked DCM genes were previously localized to Xq28 (25) (Barth syndrome), and Xp21 (26), respectively. In the latter, X-linked dilated cardiomyopathy was shown to be due to abnormalities in the dystrophin gene (27), which encodes for a cytoskeletal protein. This remains the only FDCM causing gene identified in which the function of the protein has been studied. Recently, the gene for Barth syndrome has been reported to be *G4.5*, encoding a novel protein of uncertain function (28).

In this study we provide evidence for linkage of FDCM to another locus, the long arm of chromosome 10 (10q21-10q23) in one family in which the phenotype is a pure dilated cardiomyopathy with mitral valve prolapse (MVP). Candidate genes on this portion of chromosome 10 are being evaluated currently.

Methods

Clinical evaluation. After informed consent was obtained within the guidelines of Baylor College of Medicine and Texas Children's Hospital, clinical evaluation of one family (DCM 101) was performed. Evaluation included medical and family history, physical examination, electrocardiography (12- or 15-lead), and echocardiography (M-mode, two-dimensional, Doppler, and color Doppler) as previously described (24). Diagnostic criteria for DCM were based on the World Health Organization (1) and the National Heart, Lung, and Blood Institute Workshop guidelines (5) and included echocardiographic evidence of ventricular dysfunction (left ventricular shortening fraction ≤ 27% or ejection fraction ≤ 50%) in the presence of ventricular dila-

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1. *Abbreviations used in this paper:* DCM, dilated cardiomyopathy; DMD, Duchenne muscular dystrophy; FDCM, familial DCM; LQTS, long QT syndrome; MVP, mitral valve prolapse; STR, short tandem repeat.

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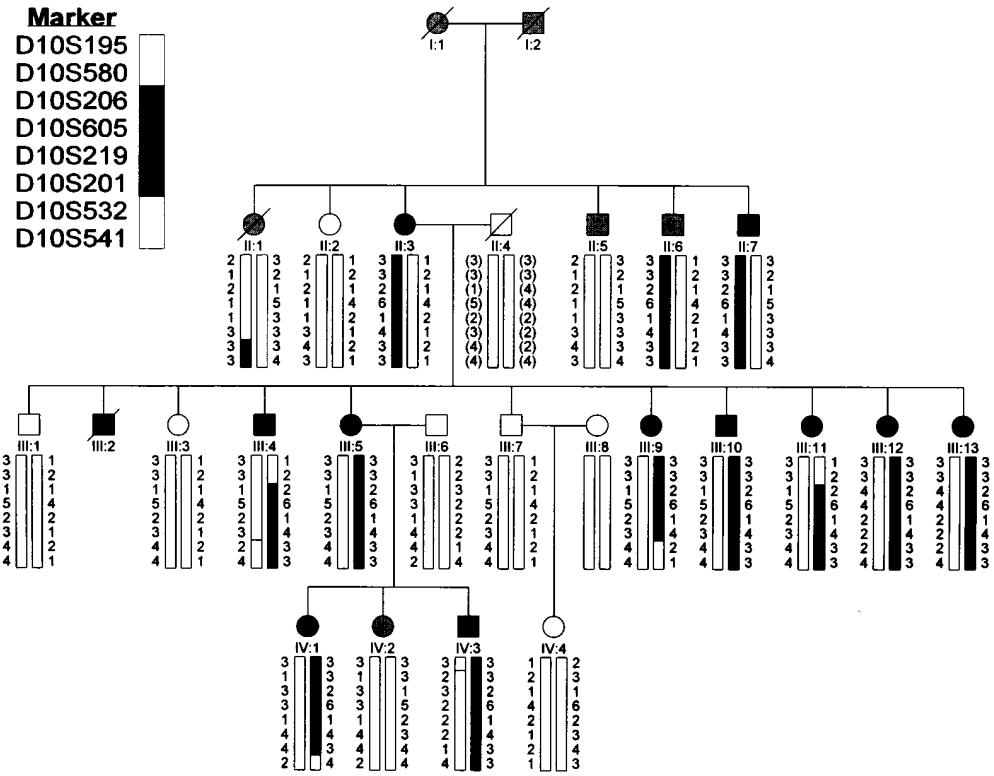

Figure 1. Pedigree structure and haplotypes for Family *DCM 101*. Females are designated by circles and males are designated by squares. Affected status is indicated by filled symbols, unaffected by unfilled symbols, and uncertain status by gray symbols. Diagonal lines indicate deceased individuals. Genotypes for markers D10S195, D10S580, D10S206, D10S605, D10S219, D10S201, D10S532, and D10S541 (centromere to telomere) are shown; inferred genotypes are shown in parenthesis. The disease-associated haplotype is indicated by black boxes; cross-over events are indicated by the lack of shading of the haplotype where expected or by horizontal lines.

Table I. Family DCM 101: Phenotype

Pedigree	Age	Age at Dx	Age at death	CHF	SCD	SF	EF	LVEDD	MR	MVP	Ventricular arrhythmias proven	Palpitations	Phenotypic status	Other comments
	yr					%	%	*mm*						
II-1	63	58	63	Y	Y	25	48	64	N	N	N	N	U	Alcoholism
II-2	75		—	N	N	32	57	47	N	N	N	N	NA	
II-3	71	54	—	Y	N	< 10	< 20	70	Y	Y	Y	Y	A	
II-4	55	50	55	Y	Y	—	40	64	N	N	N	N	NA	CAD, MI
II-5	81	—	—	N	N	?	?	?	N	N	N	N	U	No echo
II-6	85	—	—	Y	N	?	?	?	Y	N	N	N	U	No echo
II-7	78	65	—	Y	N	25	48	60	N	N	N	N	A	
III-1	48	—	—	N	N	38	66	56	N	N	N	N	NA	
III-2	23	22	23	Y	Y	—	30	66	Y	N	Y	Y	A	
III-3	46	—	—	N	N	36	62	44	N	N	N	N	NA	
III-4	44	37	—	N	N	25	48	60	Y	Y	Y	Y	A	
III-5	38	33	—	Y	N	22	44	64	Y	Y	Y	Y	A	
III-7	40	—	—	N	N	32	60	56	N	N	N	N	NA	
III-9	38	30	—	N	N	25	48	60	Y	Y	N	Y	A	
III-10	37	—	—	N	N	22	44	60	N	Y	N	Y	A	
III-11	35	—	—	N	N	23	46	60	N	Y	N	Y	A	
III-12	34	24	—	Y	N	23	46	60	Y	Y	Y	Y	A	
III-13	31	27	—	N	N	25	48	59	Y	Y	N	Y	A	
IV-1	18	17	—	N	N	25	48	56	Y	N	N	Y	A	
IV-2	15	14	—	N	N	38	66	53	N	N	N	N	U	
IV-3	14	13	—	N	N	27	49	46	N	N	N	N	A	
IV-4	23	—	—	N	N	32	60	48	N	N	N	N	NA	

Clinical phenotypes of the members of Family DCM 101. All patients were considered affected (i.e., dilated cardiomyopathy) if the shortening fraction (*SF*) was ≤ 27% or ejection fraction (*EF*) was ≤ 50%, and left ventricular end diastolic dimension (*LVEDD*) was < 2 SD above the mean for patient size. Individuals with dilated ventricles but normal SF were considered as status uncertain. Diagnostic criteria were obtained by echocardiography, cardiac catheterization, or at autopsy. *Y*, yes; *N*, no; *U*, uncertain; *NA*, not affected; *A*, affected; *CHF*, congestive heart failure; *SCD*, sudden cardiac death; *MR*, mitral regurgitation; *MVP*, mitral valve prolapse.

tation (left ventricular internal diastolic diameter > 2.7 cm/m^2 of body surface area or > 2 SD above the mean for body surface area). Normal values for echocardiographic measurements were previously determined for adults and children (29, 30). Family members in which criteria for ventricular dilatation without dysfunction was identified were classified as clinically uncertain (5). Furthermore, patients < 21-yr old were classified as uncertain if they had echocardiographic measurements within the normal range. In addition, other diseases potentially causing secondary cardiac dysfunction (i.e., coronary artery disease, myocarditis, alcohol toxicity, hypertension) were excluded. Invasive studies such as coronary angiography and endomyocardial biopsy were performed when clinically indicated. If these other disorders could not be definitively excluded, the individual was also classified as uncertain. Blood studies performed for exclusion included thyroid function studies, viral and toxoplasma titers, carnitine, iron, total iron binding capacity, and creatine kinase studies. For deceased relatives, hospital records and autopsy reports were examined. The phenotypic status was established in all cases by two echocardiographers (R.J. Gajarski and R. Pignatelli) without prior knowledge of genotype.

Genetic studies. Blood was obtained (10–12 ml) in sodium heparin tubes and lymphoblastoid cell lines were immortalized using Epstein-Barr Virus and Cyclosporin A (31). DNA extraction was performed manually as previously described (32) or with use of an automated DNA extractor (model 340A; Applied Biosystems, Foster City, CA). Short tandem repeat (STR) oligonucleotide primers within the 9q13-q22 (23), 1p1-1q1 (20), 1q32 (22), 3p25-p22 (21), and chromosome 10q (33–35) were designed and PCR performed using the sequences and conditions previously described (20–23, 33–35). All STR markers had > 70% heterozygosity. The specific chromosome 10q markers used (qcen to qter) included: D10S195, D10S580, D10S206, D10S605, D10S219, D10S201, D10S532, and D10S541 (33, 35). All PCR reactions were performed using a Stratagene Robocycler 96 Thermocycler (La Jolla, CA).

Linkage analysis. The simulated linkage power of family DCM 101 was tested using the SIMLINK (version 4.1) and MSIM Computer program (36–39). The LINKAGE version 5.2 software package was used to perform two point linkage analysis (MLINK) (40, 41), and multipoint linkage analysis was performed using the LINKMAP program. The initial penetrance level was set at 0.90 based on the number of affected individuals in an at risk sibship. Although the sibship indicated that 100% penetrance was possible, a more conservative estimate was taken in order to ensure that linkage was not missed. Penetrance was varied from 100 to 60% to provide maximal sensitivity and test for robustness of linkage. Autosomal dominant inheritance was assumed based on results of the segregation analysis. Microsatellite frequencies were set as equal (1/n). The allele frequencies for the disease and normal alleles were set at 0.0003 and 0.9997, respectively.

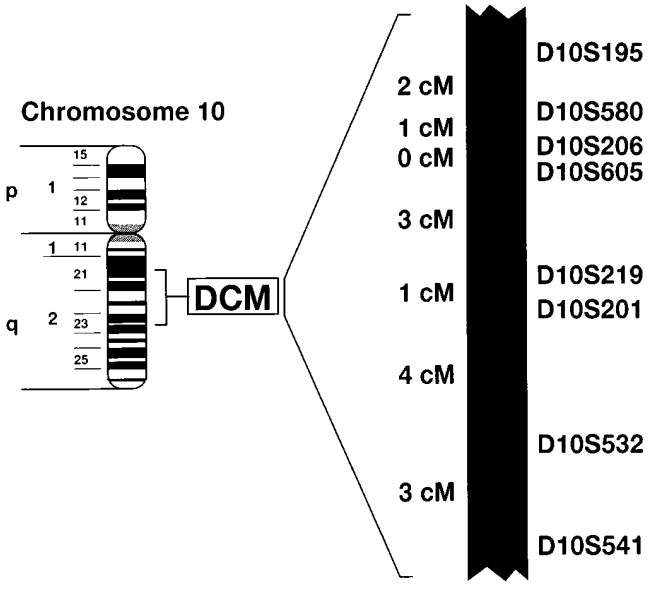

Figure 2. Ideogram of chromosome 10 (*left*) demonstrating the region of linkage (10q21-q23) in Family DCM 101. The 10q21-23 region is expanded (*right*) to show the markers used in this region and their physical distances (in centimorgans, cM) from each other. Maximal pairwise linkage occurs at markers *D10S605* and *D10S201.*

Results

Phenotypic analysis

Family DCM 101. The family (Fig. 1) was previously reported (24) and currently consists of 26 members, 12 of which are affected with FDCM (Table I). Eight of the members were normal, and the remaining six family members had uncertain status based on clinical data (patients *I:1*, *I:2*, *II:1*; Table I and Fig. 1), lack of echocardiographic information (patients *II:5*, *II:6*; Table I and Fig. 1), autopsy (patient *II:1*; Table I and Fig. 1), or young age (patient *IV:2*, Table I and Fig. 1). In eight individuals, mitral valve prolapse (MVP) was diagnosed by physical examination (i.e., evidence of mid systolic click with or without a murmur consistent with mitral regurgitation); in all cases, MVP was also found on echocardiography. Mitral regurgitation was diagnosed either by an audible mitral regurgitation murmur or by evidence of at least mild mitral regurgitation by Doppler and color Doppler echocardiographic studies. Secondary causes of DCM were excluded in all patients except II:1 in whom an alcoholic cardiomyopathy was suspected. This patient was, therefore, considered to be of uncertain status. Patient IV:2 was considered uncertain due to her young age (16 yr) since FDCM shows age related penetrance; her phenotype (and genotype), however, indicates that she is probably normal. Segregation analysis indicated that the form of FDCM in this family was inherited in an autosomal dominant manner with high penetrance (initially assumed to be 90%).

Table II. Pairwise LOD Scores: Recombination Fraction

Marker	0.0	0.1	0.2	0.3	0.4	Max LOD*
D10S195	−8.09	0.81	0.85	0.60	0.22	0.89, θ = 0.16
D10S580	−8.09	0.81	0.85	0.60	0.22	0.89, θ = 0.16
D10S206	3.31	2.76	2.15	1.45	0.66	3.31, θ = 0.00
D10S605	3.91	3.23	2.47	1.62	0.70	3.91, θ = 0.00
D10S219	3.01	2.51	1.94	1.31	0.58	3.01, θ = 0.00
D10S201	3.91	3.23	2.47	1.62	0.70	3.91, θ = 0.00
D10S532	−1.79	2.27	1.86	1.26	0.54	2.31, θ = 0.07
D10S541	−7.79	0.85	0.94	0.72	0.31	0.96, θ = 0.17

Pairwise LOD scores for Family DCM 101 with chromosome 10q markers. Two-point LOD scores were calculated assuming autosomal dominant inheritance, disease allele frequency of 0.003, and 100% penetrance. Male and female recombination frequencies were assumed to be equal. Maximum LOD score = 3.91 at θ = 0 is seen with markers D10S605 and D10S201. Recombination events occur with markers D10S580 and D10S532, narrowing the critical region to 9 cM.
*Penetrance = 100%

Table III. Maximum Experimentally Derived and Simulated LOD Scores at Different Penetrance Estimates

	Penetrance				
Marker	60%	70%	80%	90%	100%
D10S195	0.47, $\theta = 0.18$	0.56, $\theta = 0.17$	0.66, $\theta = 0.17$	0.77, $\theta = 0.16$	0.89, $\theta = 0.16$
D10S580	0.47, $\theta = 0.18$	0.57, $\theta = 0.17$	0.67, $\theta = 0.17$	0.77, $\theta = 0.16$	0.89, $\theta = 0.16$
D10S206	2.90, $\theta = 0.00$	3.00, $\theta = 0.00$	3.10, $\theta = 0.00$	3.20, $\theta = 0.00$	3.31, $\theta = 0.00$
D10S605	3.36, $\theta = 0.00$	3.49, $\theta = 0.00$	3.62, $\theta = 0.00$	3.77, $\theta = 0.00$	3.91, $\theta = 0.00$
D10S219	2.60, $\theta = 0.00$	2.70, $\theta = 0.00$	2.80, $\theta = 0.00$	2.90, $\theta = 0.00$	3.01, $\theta = 0.00$
D10S201	3.36, $\theta = 0.00$	3.49, $\theta = 0.00$	3.62, $\theta = 0.00$	3.77, $\theta = 0.00$	3.91, $\theta = 0.00$
D10S532	1.81, $\theta = 0.07$	1.93, $\theta = 0.07$	2.05, $\theta = 0.07$	2.18, $\theta = 0.07$	2.31, $\theta = 0.07$
D10S541	0.65, $\theta = 0.18$	0.72, $\theta = 0.18$	0.80, $\theta = 0.18$	0.88, $\theta = 0.17$	0.96, $\theta = 0.17$
Max simulated LOD scores at $\theta = 0.00$	3.36	3.49	3.62	3.77	3.91

Maximum experimentally derived and simulated LOD scores at different penetrance estimates, ranging from 60%–100%, to test the robustness of linkage. The maximum LOD scores range from 3.36, $\theta = 0$ at 60% penetrance to 3.91, $\theta = 0$ at 100% penetrance, which are identical to the LOD scores obtained by simulation (i.e., tight linkage).

Linkage simulation. Using the SLINK and MSIM Computer packages (36–39) to test the maximum power of linkage in this family, linkage simulation under ideal conditions was performed. A maximum LOD score = 3.77 was attained in this simulation at $\theta = 0$, penetrance = 90%, and increased to LOD = 3.91, $\theta = 0$ at penetrance = 100% (see Table III).

Linkage analysis. Using STR markers from the previously identified FDCM loci on chromosomes 1p1-1q1 (20), and 9q13-q22 (23), linkage analysis using pairwise analysis (MLINK) resulted in exclusion at both loci with LOD scores < -2, as previously reported (24). Linkage analysis using markers within and flanking 1q32 (22) and 3p25-22 (21) also excluded linkage with LOD scores < -2 (data not shown). Random genome screening was undertaken with 169 highly polymorphic microsatellite repeat sequences dispersed throughout the genome, excluding 50% of the genome. Using STR sequences from chromosome 10q21-q23, a pairwise LOD score = 3.77, $\theta = 0$, penetrance = 90% was obtained for microsatellite markers D10S605 and D10S201 (Table II); this LOD score is equal to the maximum LOD score predicted by the simulation (Table III). Recombinants were seen using primers D10S580 and D10S532 (Fig. 2, individuals *III:4* at *D10S580* and *III:9* at *D10S532*) thus defining the critical region to within 9 cM. Maximum LOD scores were obtained using a penetrance of 100% (LOD = 3.91; Table II and Table III). To test the robustness of linkage, penetrance was varied between 60%–100% with persistence of linkage (LOD = 3.36, $\theta = 0$ at 60% penetrance). Multipoint linkage analysis yielded no additional information (data not shown).

Discussion

The use of molecular genetic techniques has enabled clinicians and scientists to better understand a variety of cardiovascular disorders. In many of the familial cardiovascular disorders thus far studied, genetic heterogeneity has been demonstrated (43–44). Strikingly, however, in several of these disorders, the different genes responsible for the similar cardiac phenotype encoded proteins with distinctly similar functions. For instance, the discovery of the genes responsible for familial hypertrophic cardiomyopathy provided evidence that the presenting phenotype of these patients is due to abnormalities of the sarcomere (45). Similarly, the long QT syndrome (LQTS) phenotype has also been shown to be due to a final common pathway. In LQTS, mutations in different ion channels (potassium, sodium) (46–48) have been shown to result in the repolarization abnormalities that are the hallmark of this disease, thus confirming the long held hypothesis about the pathogenic mechanism of this disease.

Although significant progress has been made in the elucidation of the molecular pathogenesis of the cardiovascular disorders outlined above, little success had been attained for DCM. Until last year, the only FDCM genes mapped were reported in families with X-linked inheritance. Bolhuis et al. (25) reported linkage to Xq28 in patients with Barth syndrome, an X-linked cardioskeletal myopathy with neutropenia and abnormal mitochondria that presents in infancy and is rapidly fatal (49). The disease-causing gene was recently identified (*G4.5*) but the function of the protein is not known (28). Towbin et al. demonstrated linkage to the dystrophin gene at Xp21 in families with XLCM and no evidence of skeletal disease and Muntoni et al. (27) identified mutations in the dystrophin gene. In 1994, Kass and colleagues (20) were the first to map a gene for autosomal dominant FDCM, describing linkage to the 1p1-1q1 region of chromosome 1. This family, however, had an unusual clinical phenotype in which DCM is preceded by conduction abnormalities by many years. Subsequently, Olson and Keating (21) mapped a gene to 3p25-p22 in a similar family, consistent with genetic heterogeneity in this subgroup of patients with FDCM and conduction disease. Neither gene is known at the present time.

In 1995, the discovery of gene locations for FDCM in families with pure FDCM was reported. Krajinovic et al. (23) provided evidence for linkage to 9q13-q22 in three families. Schultz et al. (24) showed that genetic heterogeneity exists in pure FDCM, which was confirmed by Durand et al. (22) who found linkage to 1q32 in one family. Although these genes have not been identified, a number of candidate genes have been described, all based on the various potential pathophysiologic mechanisms of DCM. We report the discovery of the third locus for pure autosomal dominant FDCM to chromosome 10q21-q23 and provide further speculation of the dis-

ease-causing genes. In addition to FDCM, this family has associated MVP and mitral regurgitation. Three members of this FDCM family have mitral regurgitation only, two members have MVP only, and six members have both MVP and mitral regurgitation. In all cases, these family members have either affected or uncertain FDCM status. MVP is common in the normal population, affecting as many as 20% of females and 1% of males (50, 51); for this reason, it is difficult to determine whether MVP in this family with FDCM is an associated defect due to the underlying DCM-causing genetic abnormality, a separate pathologic disorder, or a completely unrelated finding that is simply common in the population. However, it has long been known that MVP occurs in patients with primary idiopathic DCM (52, 53), as well as in genetic disorders with associated DCM, such as Duchenne muscular dystrophy (DMD). Sanyal and colleagues (54) demonstrated that mitral valve prolapse and mitral regurgitation result from degeneration of the posterior papillary muscles or ventricular myocardium in these patients, causing instability of the mitral apparatus and secondary prolapse. In both idiopathic DCM and DMD-related DCM, the underlying etiology of MVP has remained elusive. However, since FDCM is a primary disorder of the ventricular myocardium due to a genetically determined protein defect (such as a cytoskeletal protein), which would also involve the papillary muscles, the DCM-causing protein defect could also be the underlying cause of associated MVP. On the other hand, mitral regurgitation without MVP is a common finding in patients with DCM due to stretching of the mitral annulus caused by the left ventricular and left atrial dilation; this is not likely to be due to the underlying gene (and ultimately protein) defect. Another possibility for the association of FDCM and MVP in this family includes a contiguous gene syndrome, although this is unlikely.

The region of interest on 10q21-q23 contains a number of candidate genes, including muscle membrane proteins (i.e., vinculin ([55]), metavinculin ([56]), actin ([57]), ankyrin ([58]), and laminin ([59]), energy-producing proteins (ATP synthase) (60, 61), and proteins responsible for energy transport (perforin) (62), which we are presently evaluating. However, based on the previously described cardiovascular diseases in which genetic heterogeneity has been clearly shown, we would speculate that in all likelihood, the genes responsible for FDCM encode proteins having similar properties (i.e., a final common pathway for development of the phenotype based on protein function). This precedent has previously been set by the information gained for a hypertrophic cardiomyopathy in which sarcomeric protein defects result in the common hypertrophic cardiomyopathy phenotype, as well as long QT syndrome where ion channel defects lead to the repolarization abnormalities that define the phenotype of this disease. Dystrophin, the gene responsible for DMD and X-linked dilated cardiomyopathy, is currently the only gene for FDCM in which the function of the protein (i.e., cytoskeletal protein) is known. Mutations in this protein have been shown to weaken the skeletal muscle membrane, making it vulnerable to necrosis by the contraction process (63, 64). We speculate that the disease-causing genes for FDCM will similarly encode cytoskeletal membrane proteins which provide mechanical support to the myocyte cell membrane. Once disrupted, these abnormal proteins would be expected to fail at supporting the membrane structure of the beating heart, ultimately leading to dilatation and dysfunction of the pump, primarily the left ventricle. Identification of new FDCM-causing genes and the responsible mutations will allow us to verify the speculation that cytoskeletal protein abnormalities underlie the resultant ventricular dilatation and dysfunction characteristic of dilated cardiomyopathies. In addition, once the genes and their mutations are identified, it is possible that pre-symptomatic diagnosis and improved therapeutic options based on the underlying cause of disease can be developed, resulting in better long term care and survival for patients with DCM and their family members who are at risk.

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