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Linkage of Familial Dilated Cardiomyopathy to Chromosome 9

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Summary

Idiopathic dilated cardiomyopathy is a heart muscle disease of unknown etiology, characterized by impaired myocardial contractility and ventricular dilatation. The disorder is an important cause of morbidity and mortality and represents the chief indication for heart transplantation. Familial transmission is often recognized (familial dilated cardiomyopathy, or FDC), mostly with autosomal dominant inheritance. In order to understand the molecular genetic basis of the disease, a large six-generation kindred with autosomal dominant FDC was studied for linkage analysis. A genome-wide search was undertaken after a large series of candidate genes were excluded and was then extended to two other families with autosomal dominant pattern of transmission and identical clinical features. Coinheritance of the disease gene was excluded for >95% of the genome, after 251 polymorphic markers were analyzed. Linkage was found for chromosome 9q13-q22, with a maximum multipoint lod score of 4.2. There was no evidence of heterogeneity. The FDC locus was placed in the interval between loci D9S153 and D9S152. Several candidate genes for causing dilated cardiomyopathy map in this region.

Introduction

Idiopathic dilated cardiomyopathy is a heart muscle disease characterized by impaired myocardial contractility, usually associated with left ventricular or biventricular dilatation. The disease is not rare (Codd et al. 1989) and represents the primary indication for heart transplantation (Manolio et al. 1992; Dec and Fuster 1994).

The etiology of dilated cardiomyopathy is still unknown (Brandeburg et al. 1981). The major advance in the study of the pathogenetic mechanisms has been the identification of a subset of cases with clear genetic transmission (familial dilated cardiomyopathy, or FDC) (Michels et al. 1985; Mestroni et al. 1990). Familial transmission, in the past considered to be rare, has been detected in 20%–25% of patients in recent controlled surveys (Michels et al. 1992; Keeling and McKenna 1994), and these data probably still underestimate its real frequency. In FDC, different modes of inheritance and some clinical characteristics suggest genetic heterogeneity.

The most frequent form of FDC is characterized by autosomal dominant pattern of transmission with age-related penetrance (Michels et al. 1992; Mestroni et al. 1994) and presents with development of ventricular dilatation and systolic dysfunction usually in the 2d–3d decade of life. The symptoms are typically related to heart failure and ventricular arrhythmia. The identification of the primary biochemical abnormality and of the disease gene for FDC has to be considered the research priority in the study of dilated cardiomyopathy (Manolio et al. 1992).

The present study reports the results of a genome-wide analysis carried out to map the localization of the disease gene responsible for autosomal dominant FDC. As a first step, a single large six-generation family was extensively evaluated, to avoid genetic heterogeneity. The analysis was then extended to two other families with autosomal dominant FDC that were selected on the basis of the same stringent diagnostic criteria.

Material and Methods

Clinical Evaluations

The diagnosis of dilated cardiomyopathy was made according to the statement of the World Health Organization/International Society and Federation of Cardiology (Brandeburg et al. 1981) and to the guidelines of the National Heart, Lung, and Blood Institute Workshop on the Prevalence and the Etiology of Idiopathic Dilated Cardiomyopathy (Manolio et al. 1992). As described elsewhere (Mestroni et al. 1994), family members were evaluated by history, physical examination, and electro-

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cardiography as well as by M-mode, cross-sectional, and Doppler echocardiography, interpreted by two independent observers. Normal values for echocardiographic measurements were determined according to standard protocols (Henry et al. 1980; American Society of Echocardiography 1989). Diagnosis of dilated cardiomyopathy was made in the simultaneous presence of (1) ejection fraction by echocardiography, radionuclide scanning or ventriculography <45%, or M-mode fractional shortening <30%, or both; and (2) an end-diastolic left ventricular diameter >2.7 cm/m^2, excluding any known cause of myocardial disease (Brandeburg et al. 1981; Manolio et al. 1992; Dec and Fuster 1994). Relatives showing signs of cardiomyopathy underwent, whenever possible, ventriculography, coronary angiography, endomyocardial biopsy, and a comprehensive arrhythmologic evaluation (Holter, signal average electrocardiogram, exercise test). In the remainder, the evaluation was completed by noninvasive techniques (Manolio et al. 1992; Dec and Fuster 1994) and review of previous hospital records. 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. In the proband of family FDC1 (IV-29), normal skeletal muscle biopsy and karyotype analysis demonstrated the absence of subclinical skeletal muscle involvement and of chromosomal aberrations, respectively.

Over the past 4 years, all affected and unaffected members of the studied families were periodically examined, to minimize the risk of misdiagnosis. At the end of follow-up (January 1995), the affection status was established without knowledge of genotype.

Genetic Studies

DNA samples from each available member of the families were extracted by standard procedures either from fresh blood samples (Miller et al. 1988) or from continuous B lymphocyte cell lines immortalized with the Epstein-Barr virus (Neitzel 1986). Polymorphic microsatellite sequences (CA repeats) with >70% of heterozygosity were used. Oligonucleotide sequences derived mainly from Généthon maps (Gyapay et al. 1994) or Group NCM (NIH/CEPH Collaborative Mapping Group 1992), except for loci D9S199 (Graw and Kwiatkowski 1993), D9S104 (Wilkie et al. 1992), and D9S66 (Kwiatkowski et al. 1992). All the oligonucleotides were synthesized by the International Centre for Genetic Engineering and Biotechnology oligonucleotide synthesis service on an Applied Biosystem 380B synthesizer. Microsatellite amplification and analyses of PCR products were carried out as described elsewhere (Krajinovic et al. 1994).

Linkage Analysis

All polymorphisms were scored without knowledge of phenotypic data and by at least two independent observers. SIMLINK computer program version 4.1 (Plougham and Boehnke 1989) was used to test the appropriate linkage power of family FDC1. Two-point and multipoint linkage analyses were performed by using the PC version of the MLINK, LODSCORE, and LINKMAP options of the LINKAGE package (version 5.1) (Lathrop et al. 1984; Lathrop and Lalouel 1984). Two loops derived by consanguineous marriages in family FDC1 were broken by MAKEPED option of the same package. The HOMOG program (version 3.3) (Ott 1991) was used to test genetic homogeneity. Family members <16 years of age were excluded from the linkage study, to avoid the low penetrance in this age group (Mestroni et al. 1994). According to the family data, the penetrance was assumed to be 0.95. An FDC gene frequency of .0003 was derived from population surveys (Codd et al. 1989; Michels et al. 1992). Microsatellite allele frequencies were calculated from the families. No sex difference in the recombination events was assumed. All computations were performed under two models, according to the phenotypic assignment of individuals IV-24 and V-24 of FDC1 and individual III-1 of FDC3 as unknown or as affected, respectively.

In order to test the stability of the obtained lod values, the maximum two-point lod scores were recalculated applying the sensitivity test (Hodge and Greenberg 1992), varying the penetrance from 100% to 75%, or setting marker allele frequencies to equal. The support interval (90% confidence limits) was calculated using the "1-unit-down" method (Ott 1991). Because of the computer/software constraints, the number of alleles at each locus was reduced for multipoint analyses, without loss of information with respect to FDC gene.

Results

Eighty members of a single family with FDC, identified in 1987, were investigated. The selection criteria were the presence of dilated cardiomyopathy with autosomal dominant pattern of transmission, the absence of any clinical or histological sign of skeletal muscle involvement, and the sufficient informativeness for a linkage study (FDC1, fig. 1*A*). Full invasive evaluation was performed in nine patients. In the remaining patients, coronary artery disease was excluded by clinical criteria and noninvasive testing. Thirteen members of family FDC1 were considered to be affected. Family members with subclinical cardiac impairment, such as isolated frequent and repetitive ventricular arrhythmias (FDC1 V-24) or left ventricular dilatation and dysfunction in the presence of arterial hypertension (FDC1 IV-24: diastolic pressure repeatedly 120 mmHg), were con-

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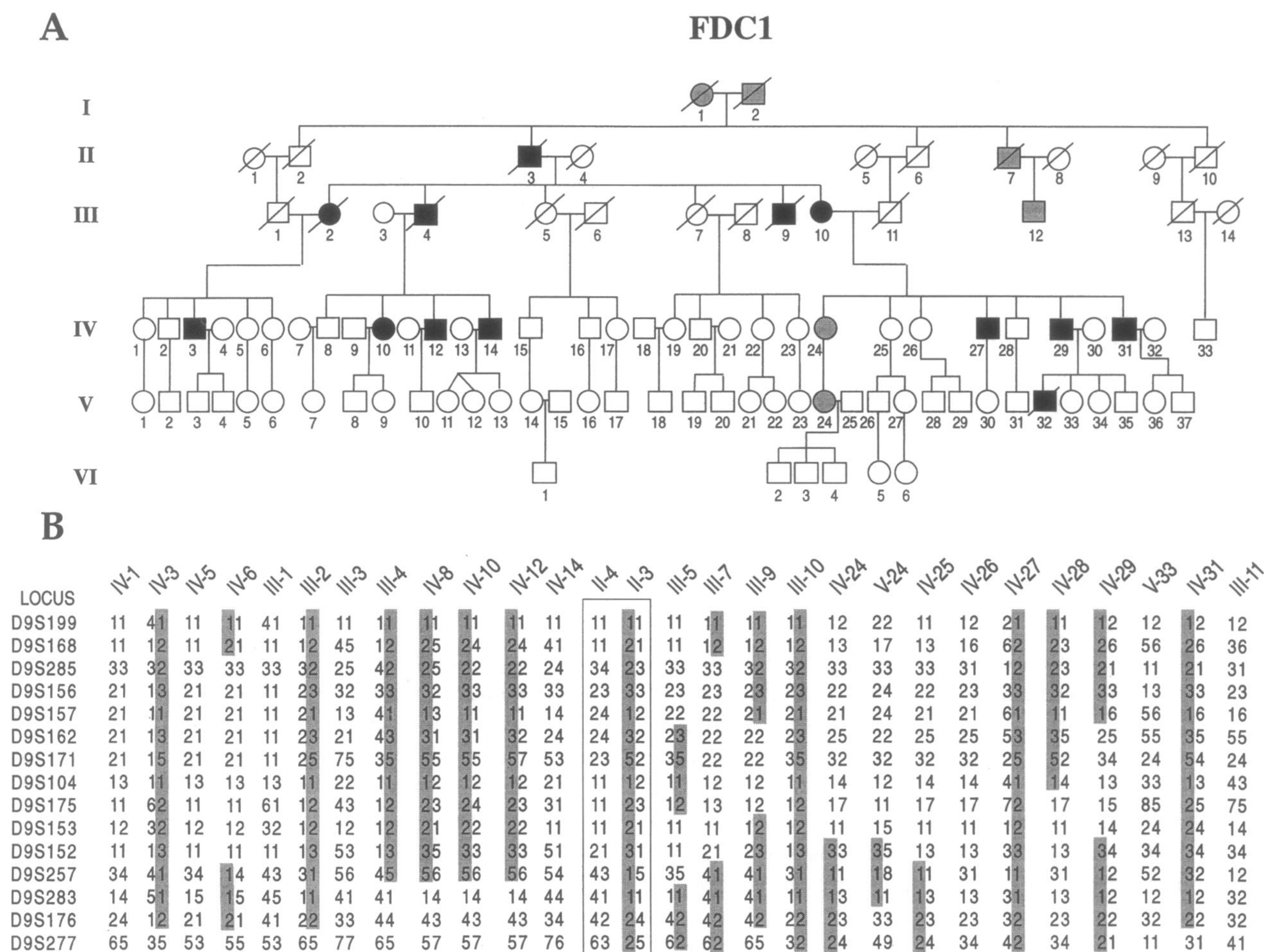

LOCUS	IV-1	IV-3	IV-5	IV-6	III-1	III-2	III-3	III-4	IV-8	IV-10	IV-12	IV-14	II-4	II-3	III-5	III-7	III-9	III-10	IV-24	V-24	IV-25	IV-26	IV-27	IV-28	IV-29	V-33	IV-31	III-11
D9S199	11	41	11	11	41	11	11	11	11	11	11	11	11	11	11	11	11	11	12	22	11	12	21	11	12	12	12	12
D9S168	11	12	11	21	11	12	45	12	25	24	24	41	11	21	11	12	12	12	13	17	13	16	62	23	26	56	26	36
D9S285	33	32	33	33	33	32	25	42	25	22	22	24	34	23	33	33	32	32	33	33	33	31	12	23	21	11	21	31
D9S156	21	13	21	21	11	23	32	33	32	33	33	33	23	33	23	23	23	23	22	24	22	23	33	32	33	13	33	23
D9S157	21	11	21	21	11	21	13	41	13	11	11	14	24	12	22	22	21	21	21	24	21	21	61	11	16	56	16	16
D9S162	21	13	21	21	11	23	21	43	31	31	32	24	24	32	23	22	22	23	25	22	25	25	53	35	25	55	35	55
D9S171	21	15	21	21	11	25	75	35	55	55	57	53	23	52	35	22	22	35	32	32	32	32	25	52	34	24	54	24
D9S104	13	11	13	13	13	11	22	11	12	12	12	21	11	12	11	12	12	11	14	12	14	14	41	14	13	33	13	43
D9S175	11	62	11	11	61	12	43	12	23	24	23	31	11	23	12	13	12	12	17	11	17	17	72	17	15	85	25	75
D9S153	12	32	12	12	32	12	12	12	21	22	22	11	11	21	11	11	12	12	11	15	11	11	12	11	14	24	24	14
D9S152	11	13	11	11	11	13	53	13	35	33	33	51	21	31	11	21	23	13	33	35	13	13	33	13	34	34	34	34
D9S257	34	41	34	14	43	31	56	45	56	56	56	54	43	15	35	41	41	31	11	18	11	31	11	31	12	52	32	12
D9S283	14	51	15	15	45	11	41	41	14	14	14	44	41	11	11	41	41	11	13	11	13	13	31	13	12	12	12	32
D9S176	24	12	21	21	41	22	33	44	43	43	43	34	42	24	42	42	42	22	23	33	23	23	32	23	22	32	22	32
D9S277	65	35	53	55	53	65	77	65	57	57	57	76	63	25	62	62	65	32	24	49	24	34	42	34	21	11	31	41

Figure 1 *A*, Pedigree of family FDC1, consistent with autosomal dominant inheritance. Individuals are indicated by generation and pedigree number. Affected status is indicated by solid symbols, unaffected status is indicated by clear symbols, and unknown status is indicated by stippled symbols. *B*, Haplotypes of the key members of FDC1, resulting from the analysis of 15 informative microsatellite markers located on chromosome 9 (Gyapay et al. 1994). The gray boxed areas represent the haplotype cosegregating with the disease. The haplotypes of individuals II-3 and II-4 are inferred.

sidered of unknown status for linkage analysis. Finally, patients with other defined cardiac affections, in particular with clinical and laboratory evidence of ischemic heart disease, were considered as unaffected.

Two other kindreds with autosomal dominant pattern of transmission (FDC2 and FDC3, fig. 2*A*) were subsequently identified from a group of 19 families with dilated cardiomyopathy; the remaining 17 families were excluded from linkage study because of insufficient informativeness, different pattern of transmission, or different clinical characteristics. Diagnosis of dilated cardiomyopathy and clinical evaluation were made according to the same criteria used for family FDC1, identifying eight affected and three unaffected relatives in families FDC2 and FDC3. One case (III-1) of family FDC3, with isolated left ventricular dilatation, was considered as unknown. The groups of affected and unaffected members enrolled in the study did not differ significantly in age and sex distribution.

The linkage study was initiated in kindred FDC1, which had been proved to have an appropriate linkage power (Krajinovic et al. 1994). Random screening was performed with 251 highly polymorphic microsatellite sequences dispersed throughout the genome. For all tested markers, two-point lod scores were calculated and exclusion maps were constructed. The distance between markers was based on Généthon microsatellite maps (Gyapay et al. 1994). The sum of the unexplored regions located between two adjacent excluded intervals, and ⩽5–10 cM, was ∼150 cM. Thus, before establishing linkage, ∼95% of the genome of 21 autosomes was excluded (Gyapay et al. 1994).

The first result suggestive of linkage to chromosome 9 was obtained by analyzing a dinucleotide repeat at the

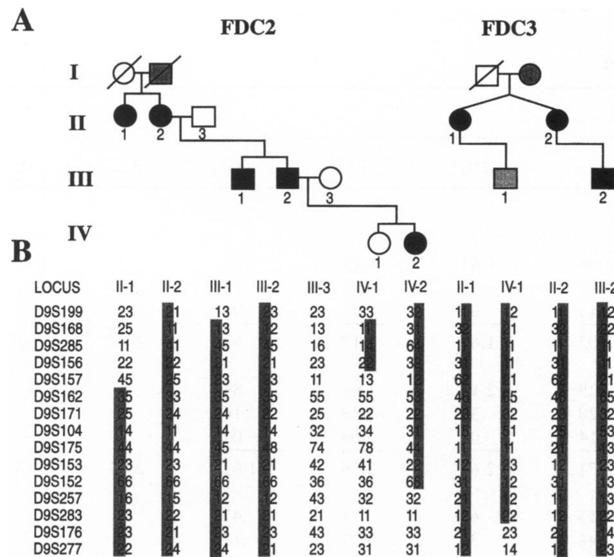

Figure 2 *A*, Pedigree structure of two families with autosomal dominant FDC linked to chromosome 9. *B*, Haplotypes. Symbols are as described in fig. 1.

D9S156 locus (Gyapay et al. 1994). Seventeen additional markers spanning the entire chromosome 9 were then tested, and, finally, the two smaller families with the same clinical features (FDC2 and FDC3) were included in the study to provide additional evidence. Linkage analysis revealed significant two point lod scores at two loci on chromosome 9, D9S153 and D9S152 (Gyapay et al. 1994). Cumulative and by family pairwise lod scores for 15 markers on chromosome 9 are presented on table 1*A*. The maximum cumulative two-point lod scores were obtained for loci D9S153 (Z_{max} = 3.69; θ = .08) and D9S152 (Z_{max} = 3.21; θ = .09).

When individuals FDC1 IV-24, V-24 and FDC3 III-1 with subclinical cardiac impairment were considered as affected, linkage was confirmed for the two loci D9S153 (Z_{max} 3.18; θ = .11) and D9S152 (Z_{max} 3.46; θ = .08). In particular, a significant lod score of 3.18 (θ = .09) was obtained with the single large kindred FDC1 in D9S152 (table 1*B*).

Lod score values remained significantly positive (⩾3) also after modifying the penetrance from 100% to 75% as well as after changing allele marker frequencies to equal. Changes in the affection status, when the sensitivity test was performed (Hodge and Greenberg 1992), did not affect the lod values. The null hypothesis of locus homogeneity (Ott 1991) was confirmed (*P* = .000041).

The reconstruction of the haplotypes for 15 markers of chromosome 9 was performed by minimizing the recombination events between the markers (fig. 1*B* and fig. 2*B*). Obligate recombination events in three affected individuals from family FDC1 (III-4, IV-29, and IV-31) and one from family FDC2 (IV-2) were identified at the loci D9S153 and D9S257, thus setting the boundaries for the disease locus. Telomeric and centromeric crossovers that occurred in the other individuals are outside of this region. The genotype of unaffected individual IV-8 (42 years old) is concordant with disease status for both D9S153 and D9S152 loci. This divergence could derive from phenotypic misclassification, recombination events between the markers and the disease gene, or reduced penetrance. Considering the reduced and age-related penetrance of FDC, the latter hypothesis seems to be most likely one. Affected individual IV-14 of family FDC1 was recombinant for the markers within the interval D9S153-D9S257. Although the possibility of phenocopies could not be excluded, this is not likely to be the case, since this patient was studied with full invasive and noninvasive examinations and showed the typical features of dilated cardiomyopathy. Because segregation of the disease is suggestive of monogenic disorder, an epistatic influence of some other gene(s) appears equally unlikely, though it could not be completely ruled out. A third, most likely, explanation seems to be the occurrence of a double recombination within this interval. This hypothesis is supported by the high level of interference predicted in the region 9cen-9q32 (Attwood et al. 1994).

To determine the most probable location for the disease locus, multipoint linkage analysis was performed with markers D9S175, D9S153, D9S152, D9S257, and D9S283, using the LINKMAP program. A multipoint lod score value of 4.2 was obtained. This result is consistent with the two-point and haplotype analyses, leading to the localization of the disease locus between D9S153 and D9S152 markers (fig. 3), under either stringent or relaxed models of phenotypic assignment.

Discussion

The application of molecular genetic techniques and linkage analysis for the identification of the gene (or genes) for dilated cardiomyopathy has been hampered so far by high incidence of premature mortality, small family sample size, absence of early clinical markers, age-related penetrance, and, finally, evidence of genetic heterogeneity. In a series of families with dilated cardiomyopathy under prospective evaluation, we had the opportunity to identify a large kindred, which enabled us to undertake genetic analysis on a single family, thus avoiding genetic heterogeneity. Initially, several candidate genes were excluded by linkage analysis (Krajinovic et al. 1994). Subsequently, a genome-wide random screening with anonymous polymorphic marker was carried out. This approach indicated a high probability of linkage of FDC to the long arm of chromosome 9 as well as the complete exclusion of linkage for ~95% of the other 21 autosomes. The extension of the analysis

Table 1

Cumulative and Familial Lod Scores for 15 Markers of Chromosome 9

Status, Marker, and Kindred	Z at θ = .00	.01	.05	.10	.20	.30	.40	Z_{max}	θ_{max}
A.									
D9S156:									
FDC1	2.46	2.42	2.27	2.06	1.63	1.15	.61		
FDC2	−.53	−.47	−.30	−.17	−.06	−.01	.00		
FDC3	.20	.20	.17	.13	.08	.03	.01		
Cumulative	2.13	2.15	2.14	2.02	1.65	1.17	.62	2.16	.02
D9S157									
FDC1	−1.40	1.20	2.03	2.29	2.16	1.63	.84		
FDC2	−2.30	−.53	.08	.28	.36	.39	.16		
FDC3	.19	.18	.16	.14	.09	.04	.01		
Cumulative	−3.51	.85	2.27	2.71	2.61	2.06	1.01	2.76	.13
D9S162:									
FDC1	−17.76	−4.13	−1.29	−.15	.63	.71	.47		
FDC2	.53	.53	.50	.46	.38	.27	.15		
FDC3	.16	.15	.13	.11	.07	.03	.01		
Cumulative	−17.07	−3.45	−.66	.42	1.08	1.01	.63	.11	.24
D9S171:									
FDC1	−15.45	−4.39	−1.52	−.35	.50	.65	.45		
FDC2	−.86	−.81	−.62	−.45	−.22	−.09	−.02		
FDC3	.20	.19	.17	.14	.08	.04	.01		
Cumulative	−16.11	−5.01	−1.97	−.66	.36	.60	.44	.6	.30
D9S104:									
FDC1	−6.31	−1.91	−.79	−.34	−.01	.07	.06		
FDC2	.41	.41	.40	.39	.34	.26	.15		
FDC3	.24	.23	.19	.14	.06	.02	.00		
Cumulative	−5.66	−1.27	−.20	.19	.39	.35	.21	.39	.22
D9S175:									
FDC1	−7.98	−1.34	.60	1.27	1.53	1.26	.70		
FDC2	.74	.72	.67	.60	.46	.31	.16		
FDC3	.16	.15	.11	.08	.02	.00	.00		
Cumulative	−7.08	−.47	1.38	1.95	2.01	1.57	.86	2.08	.15
D9S153[a]:									
FDC1	−5.37	1.49	2.57	2.76	2.47	1.80	.92		
FDC2	1.05	1.02	.93	.82	.60	.38	.18		
FDC3	.13	.12	.11	.08	.05	.02	.01		
Cumulative	−4.19	2.63	3.61	3.66	3.12	2.20	1.11	3.69	.08
D9S152[b]:									
FDC1	−.74	2.11	2.79	2.92	2.61	1.93	.99		
FDC2	.27	.26	.23	.19	.12	.06	.01		
FDC3	.13	.12	.11	.09	.05	.02	.01		
Cumulative	−.34	2.49	3.13	3.20	2.78	2.01	1.01	3.21	.09
D9S257:									
FDC1	−18.40	−6.13	−2.95	−1.49	−.21	.25	.28		
FDC2	−4.81	−1.00	−.38	−.16	−.04	−.01	−.01		
FDC3	−.09	−.09	−.07	−.06	−.03	−.01	.00		
Cumulative	−23.30	−7.22	−3.40	−1.71	−.28	.23	.27	.29	.36
B.									
D9S153:									
FDC1	−5.09	−.21	1.57	2.06	2.07	1.58	.82		
FDC2	1.05	1.02	.93	.82	.60	.38	.18		
FDC3	.43	.42	.36	.29	.17	.08	.02		
Cumulative	−3.61	1.23	2.86	3.17	2.84	2.04	1.02	3.18	.11
D9S152:									
FDC1	−.44	2.41	3.07	3.18	2.81	2.07	1.07		
FDC2	.27	.26	.23	.19	.12	.06	.01		
FDC3	.11	.10	.09	.07	.04	.02	.01		
Cumulative	−.06	2.77	3.39	3.44	2.97	2.15	1.09	3.46	.08

NOTE—Lod scores calculated using two different models of phenotypic assignment: A. assuming individuals with subclinical cardiac impairment as unknown; B. as affected (see text).

[a] Ninety percent confidence limits: .011–.25.

[b] Ninety percent confidence limits: .006–.27.

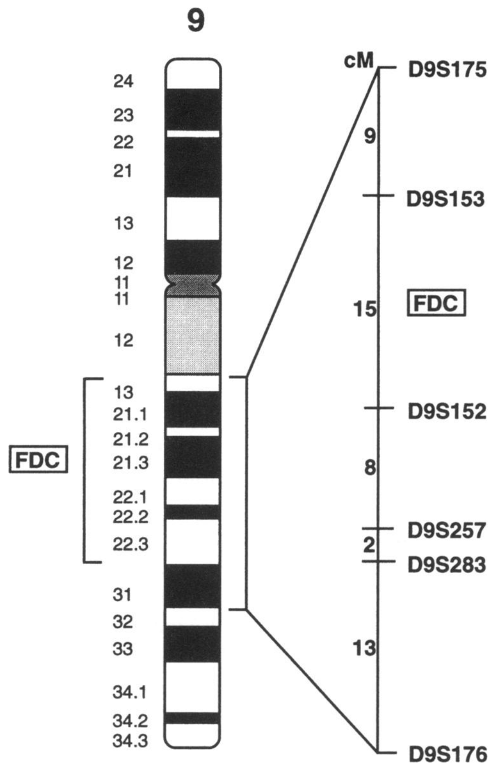

Figure 3 Ideogram of chromosome 9 showing approximate location of FDC gene. The genetic map shows sex-averaged recombinant fractions between adjacent markers. The FDC locus is genetically mapped between D9S153 and D9S152. The order and distances are based on data from Généthon (Gyapay 1994) and CHLC (1994).

to two other families, selected according to the same clinical and genetic characteristics, confirmed the evidence of linkage to this region of chromosome 9. According to multipoint linkage and haplotype analyses, the gene for FDC in these families lies in a region of ~15 cM between D9S153 and D9S152 loci (CHLC 1994). The approximate location of this interval corresponds to region 9q13-q22 (fig. 3). It is interesting that individuals classified as unknown, because of minor signs of cardiac impairment such as isolated ventricular dilatation, were found to carry the disease genotype.

Studies on systemic diseases associated with dilated cardiomyopathy indicate that genes encoding for proteins with different functions can lead to the same ultimate phenotype of myocardial dilatation and dysfunction (Dec and Fuster 1994; Kelly and Strauss 1994). According to these observations, the localization of FDC gene suggests that several genes could be considered as possible candidates for causing the disease. Among them are the gene for Friedreich ataxia (9q13-q21.1) (Chamberlain et al. 1988; Fujita et al. 1991), a severe neurodegenerative disease with autosomal recessive transmission frequently associated with dilated cardiomyopathy (Perloff et al. 1988), and the gene for the cAMP-dependent protein kinase (9q13), which regulates the calcium-channel ion conductance in the heart (Catterall 1988).

However, the most attractive candidate is the gene coding for tropomodulin (9q22) (Sung et al. 1991). This novel tropomyosin regulatory protein, localized at or near the pointed end of actin filaments, inhibits tropomyosin binding to actin and is believed to interact with the membrane cytoskeleton. Preliminary data report the presence of tropomodulin also in myocardial cells (Sung and Lin 1994).

Mapping a locus for dilated cardiomyopathy represents the first step for the identification of the disease gene, of its mutations, and of the biochemical dysfunction leading to the disorder. The correlation of linkage data with the clinical and histological features will greatly increase the understanding of this disease and will clarify whether subclinical cardiac abnormalities, such as isolated enlargement of the left ventricle, isolated arrhythmias, or ventricular dysfunction in the presence of hypertension, may actually represent early or incomplete manifestations of the disease. Moreover, the detection of an FDC locus allows the determination of the gene-carrier status within families and the prevention of the progression of the disease in asymptomatic carriers. Finally, it can be expected in the future that treatments based on the understanding of the molecular mechanisms of the disease will replace the current symptomatic approach and avoid the need for heart transplantation.

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