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Report

A New Locus for Autosomal Dominant Dilated Cardiomyopathy Identified on Chromosome 6q12-q16

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Dilated cardiomyopathy (DCM) is a heart-muscle disease characterized by ventricular dilatation and impaired heart contraction and is heterogeneous both clinically and genetically. To date, 12 candidate disease loci have been described for autosomal dominant DCM. We report the identification of a new locus on chromosome 6q12-16 in a French family with 9 individuals affected by the pure form of autosomal dominant DCM. This locus was found by using a genomewide search after exclusion of all reported disease loci and genes for DCM. The maximum pairwise LOD score was 3.52 at recombination fraction 0.0 for markers D6S1644 and D6S1694. Haplotype construction delineated a region of 16.4 cM between markers D6S1627 and D6S1716. This locus does not overlap with two other disease loci that have been described in nonpure forms of DCM and have been mapped on 6q23-24 and 6q23. The phospholamban, malic enzyme 1–soluble, and laminin-α4 genes were excluded as candidate genes, using single-strand conformation polymorphism or linkage analysis.

Dilated cardiomyopathy (DCM) is a heart-muscle disease characterized by ventricular dilatation and impaired systolic contraction leading to congestive heart failure and sudden death. The disease appears to be familial in 20%–30% of patients (Keeling et al. 1995; Grünig et al. 1998) and is transmitted in an autosomal dominant manner in 66% of patients (Mestroni et al. 1999*b*). Familial DCM exhibits both clinical variability and genetic heterogeneity. Three disease loci have been linked to autosomal dominant pure DCM: 1q32 (*CMD1D* [MIM 601494], Durand et al. 1995), 2q31 (*CMD1G* [MIM 604145], Siu et al. 1999), and 9q13-22 (*CMD1B* [MIM 600884], Krajinovic et al. 1995). Mutations in cardiac actin, desmin, and δ-sarcoglycan genes mapped on 15q14, 2q35, and 5q33, respectively, have been identified in the disease by using a candidate-gene approach (Olson et al. 1998; Li et al. 1999; Tsubata et al. 2000). Loci for DCM associated with conduction-system disease have been mapped on chromosome 1q21.2, with mutations reported in the *LMNA* (lamin A/C) gene (*CMD1A* [MIM 115200]) (Kass et al. 1994; Fatkin et al. 1999; Brodsky et al. 2000), 2q14-22 (*CMD1H* [MIM 604288]) (Jung et al. 1999), and 3p22-p25 (*CMD1E* [MIM 601154]) (Olson and Keating 1996). Two disease loci—on chromosomes 1q21.2, with mutations in the *LMNA* gene (*CMD1A*, Brodsky et al. 2000), and 6q23 (*CMD1F* [MIM 602067], Messina et al. 1997)—have also been described in DCM associated with skeletal-muscle abnormalities. DCM associated with mitral valve prolapse has been mapped on 10q21 (*CMD1C* [MIM 601493]) (Bowles et al. 1996), and a chromosomal locus for DCM associated with sensorineural hearing loss has been reported on 6q23-24 (*CMD1J*) (Schönberger et al. 2000).

We report results of linkage analysis of a French family in which affected individuals in three successive generations express the pure form of DCM. Family members

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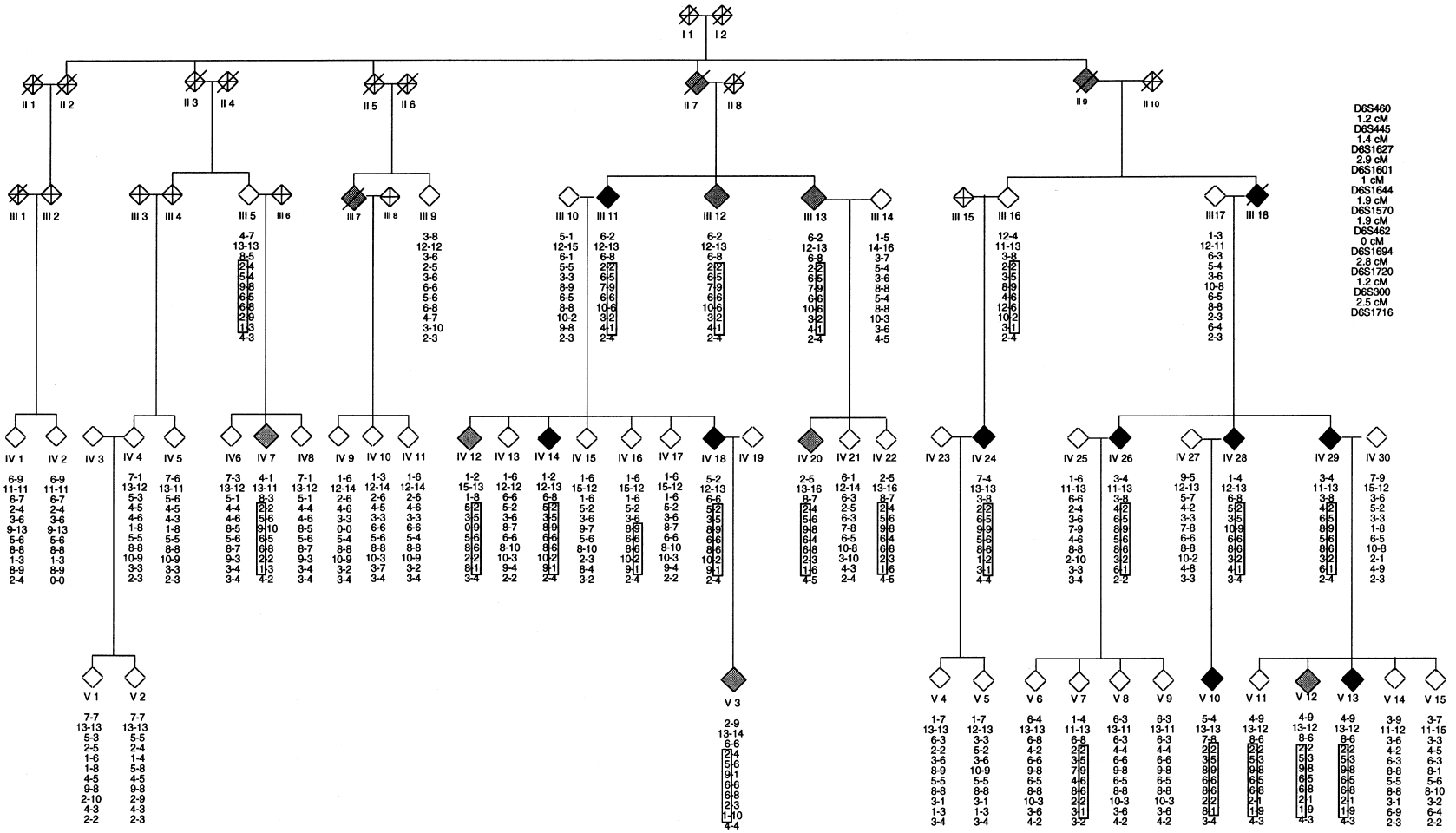

Figure 1 Pedigree showing haplotype reconstruction for chromosome 6q markers in a French family affected by autosomal dominant DCM. 0 = uncertain interpretation of genotypes even after several independent experiments. Disease haplotype is boxed. Individuals with no clinical data are indicated by a cross. Blackened symbols indicate affected individuals, unblackened symbols indicate asymptomatic individuals, and gray symbols indicate individuals with unknown DCM status.

Table 1

Clinical Data on Carriers of the Disease Haplotype

Subject	DCM Phenotype	Sex/Age (years)[a]	BSA (m^2)	NYHA Class	LVEDD (mm)	LVESD (mm)	EF (%)	ECG Finding	Comment
III5	H	M/76	1.83	I	<55	NA	>55		
III11	A	F/66	1.58	I	51	40	43	VPB	MR2/4
III12	U	F/64	1.48	II	NA	NA	42		Isolated LV dysfunction
III13	U	M/58	2.01	II	65	52	40	iLBB	DCM but CAD
III16	H	F/84	1.39	I	42	27	66	Left QRS axis	MVP; MR3/4
IV7	U	F/43	1.50	I	52	34	63		Isolated LV dilatation
IV12	U	M/45	2.03	I	56	39	57	iRBB	Isolated LV dilatation
IV14	A	M/33	1.82	I	58	46	42		
IV16	H	F/39	1.85	I	54	36	61		
IV18	A	F/40	1.78	II	60	49	37	VPB	
IV20	U	F/35	2.09	II	50	35	57		VO_2 = 19 ml/kg/min; CHF
IV22	H	M/28	2.14	I	57	40	56		
IV24	A	M/60	1.95	I	68	58	30	iLBB, VPB	
IV26	A	F/55	1.61	II	73	60	36	VPB	
IV28	A	F/48	1.68	II	53	45	32		
IV29	A	F/55	1.55	I	54	40	50		
V3	U	M/19	1.96	I	52	40	48	LAH	Isolated mild LV dysfunction
V7	H	M/34	1.90	I	54	36	61		
V10	A	M/17	1.88	I	57	46	39		
V11	H	F/34	1.60	I	49	31	66		
V12	U	M/37	2.11	I	59	40	60	iLBB	Isolated LV dilatation
V13	A	F/29	1.75	II	78	58	49	iLBB	

NOTE.— A = affected; BSA = body surface area; CAD = coronary artery disease; CHF = congestive heart failure; EF = ejection fraction; H = healthy carrier; iLBB = incomplete left bundle branch block; iRBB = incomplete right bundle branch block; LAH = left atrium hypertrophy; LV = left ventricular; LVEDD = LV end diastolic diameter; LVESD = LV end systolic diameter; MR = mitral regurgitation; MVP = mitral valve prolapse; NA = data not available; VPB = ventricular premature beats (≥1 triplet); U = unknown status. VO_2 = maximal oxygen uptake during exercise.

[a] Age at genetic inquest and clinical evaluation.

received clinical evaluation, including electrocardiogram (ECG) and echocardiography. Coronary angiography was performed in the proband and in those relatives who were suspected of having ischemic heart disease. The diagnosis of DCM was based on major and minor criteria established in a European collaboration (see details in Mestroni et al. 1999*a*). A subject's status was considered "unknown" if mild abnormalities or confounding factors, such as coronary artery disease, were present. A simplified pedigree of the family is presented in figure 1. Clinical data of 22 subjects carrying the disease haplotype are summarized in table 1. Of the nine subjects who were diagnosed as phenotypically affected by DCM, five had previously reported New York Heart Association (NYHA) class III dyspnea, and three had significant ventricular premature beats (with triplets). Seven subjects carrying the common haplotype were considered to have unknown status in the linkage analysis, because of the presence of mild cardiac abnormalities that included isolated left ventricular (LV) diameter enlargement (individuals IV7, IV12, and V12), isolated low ejection fraction (individuals III12 and V3), significant coronary artery disease that could interfere with the diagnosis of DCM (individual III13), and congestive heart failure (individual IV20). No subject had a conduction defect or skeletal muscle abnormalities at clinical examination.

Blood samples from 51 persons were obtained, and genomic DNA was extracted using standard procedures,

Table 2

Pairwise LOD Scores for 11 Markers on Chromosome 6

	LOD SCORE AT θ =[b]			
MARKER[a]	.0	.01	.05	.1
D6S460	−.10	.84	1.29	1.30
D6S445	2.56	2.52	2.34	2.10
D6S1627	−1.39	.86	1.34	1.37
D6S1601	1.28	1.26	1.16	1.03
D6S1644	3.53	3.47	3.20	2.84
D6S1570	3.03	2.98	2.79	2.54
D6S462	1.83	1.80	1.65	1.47
D6S1694	3.52	3.46	3.23	2.93
D61720	3.30	3.25	3.01	2.70
D6S300	2.77	2.72	2.51	2.24
D6S1716	−1.21	.61	1.13	1.20

[a] Markers are shown in order from centromere to telomere, according to Dib et al. (1996).

[b] Maximum LOD scores are underlined.

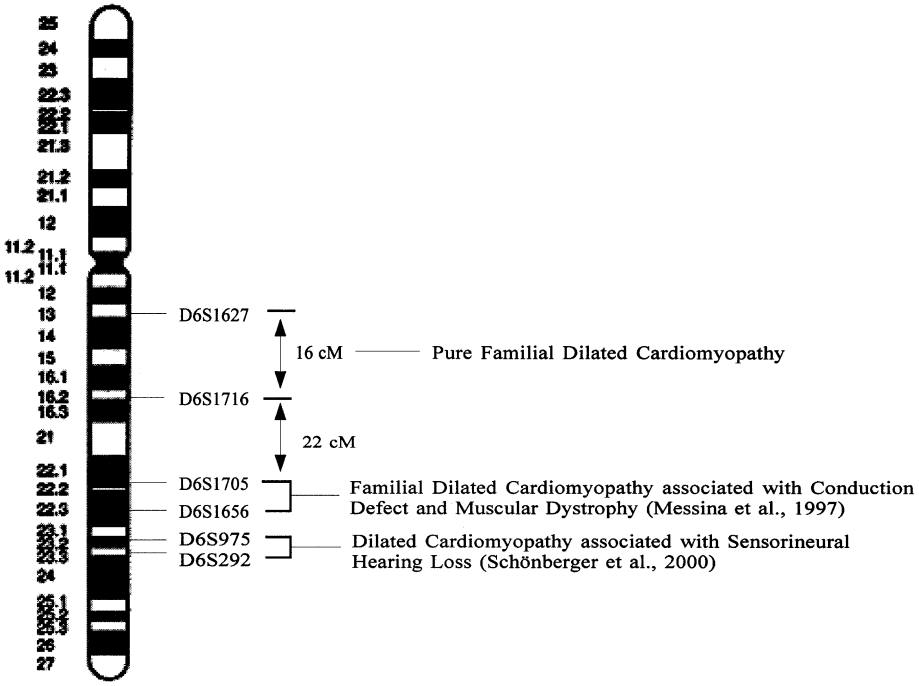

Figure 2 Ideogram of chromosome 6 with approximate location of DCM loci and flanking markers

in collaboration with the Généthon Bank. Informed consent was obtained from all participants, in accordance with requirements of the Pitié-Salpêtrière hospital ethics committee. To map the disease gene, we performed a genomewide scan with 342 fluorescent microsatellite markers selected from the Généthon human linkage map (Dib et al. 1996), which covers the entire human genome with a resolution of ~10 cM. Markers were amplified by PCR (Dib et al. 1996) and were separated on an automatic ABI 377 DNA sequencer before analysis with the GENESCAN version 2.2 and GENOTYPER version 2.0 software (Applied Biosystems).

Pairwise calculations were performed with MLINK version 5.2, under an autosomal dominant model. The allele frequencies of the microsatellite markers were set as equal ($1/n$), and frequencies for the disease and normal allele were set at .0003 and .9997, respectively (Krajinovic et al. 1995). No sex difference was considered. Penetrance of the disease, calculated according to the method of Johnson et al. (1996), was estimated at 60% (Johnson et al. 1996; Mangin et al. 1999). After exclusion of all known loci, a scan of the entire autosomal genome was performed, allowing us to exclude ~90% of the genome. Positive pairwise LOD scores (Z) were obtained for chromosomes 2, 10, and 13, but the loci were excluded by additional marker genotyping and haplotype reconstruction. The cardiac actin and desmin loci, which mapped on 15q14 and 2q35, respectively, were excluded by both genotyping and SSCP analysis of coding regions (cardiac active gene [J00070, J00071, J00072, and J00073] and desmin gene [M58168]) (Tesson et al. 2000). The lamin A/C gene was also excluded by genotyping and SSCP analysis of the entire coding sequence (L12399, L12400, and L12401). Haplotype reconstruction showed that all affected subjects, as well as all individuals with unknown status, shared a common haplotype on chromosome 6, between markers D6S1627 and D6S1716 (fig. 1). The candidate interval corresponds to a 16.4-cM region localized on chromosome 6q12-16. Using the parameters described earlier in this paragraph, we obtained maximum pairwise LOD scores of 3.53 and 3.52 with markers D6S1644 and D6S1694, respectively, at recombination fraction (θ) = 0.0 (table 2). The pairwise LOD scores for both markers remained significant, at $\theta = 0.0$, when penetrance was estimated at 75% and at 90% (the pairwise LOD score was 3.68 at 75% penetrance and was 3.48 at 90% penetrance for marker D6S1644, and the respective LOD scores were 3.35 and 2.77 for marker D6S1694). Results obtained using the published allele frequencies of the CEPH families (Dib et al. 1996) showed a pairwise LOD score of 3.98 and 3.41 for markers D6S1644 and D6S1694, respectively. When all individuals with unknown status were considered as affected, the pairwise LOD score for both markers reached 6.5 at $\theta = 0.0$, using an estimated penetrance of 60% and equal allele frequencies.

The presence of five clinically unaffected adults (subjects III5, III16, IV22, V7, and V11), who were 28–84 years old and who carried the entire disease haplotype,

points out the incomplete penetrance of the disease in this family. Subject IV16 carried the partial disease haplotype yet was asymptomatic and had strictly normal results on cardiovascular examination, ECG, and echocardiography (table 1). These observations are in agreement with those reported by Mangin et al. (1999), which showed an incomplete age-related penetrance in 13 unrelated families. The present family exhibits no significant sex-related penetrance. Individual III16 is known to have transmitted the disease haplotype, because one of his children (IV24) was clinically affected. Furthermore, seven individuals (III12, III13, IV7, IV12, IV20, V3, and V12) classified as having unknown status were also carriers of the disease haplotype, suggesting that the minor abnormalities were a mild form of the disease. The incomplete penetrance of the disease within this family and the heterogeneous expression suggest the involvement of other factors, such as modifying genes and/or environmental factors, in the phenotypic expression. Similar results have been observed in familial hypertrophic cardiomyopathy, which is also characterized by highly incomplete penetrance (Charron et al. 1997; Moolman et al. 2000).

According to the Généthon linkage map, this third locus on chromosome 6q is localized in a centromeric region 22 cM away from the disease locus described by Messina et al. (1997) and 29 cM away from the locus reported by Schönberger et al. (2000). Linkage analysis using markers D6S262 and D6S457, both localized in the telomeric boundary of the intervals described by these authors, allowed us to exclude the present locus (Z at $\theta < -2$) from these distal intervals (fig. 2).

The disease interval on chromosome 6q12-16 reported here contains known genes encoding collagen IX-α-1 polypeptide (*COL9A1* [MIM 120210]), myosin VI (*MYO6* [MIM 600970]), vascular endothelial growth factor (*VEGF* [MIM 192240]), malic enzyme cytoplasmic (*ME1* [MIM 154250]) and several other genes encoding anonymously expressed sequence tags. In addition, the genes encoding cardiac phospholamban (*PLN* [MIM 600133]) and laminin-α4 (*LAMA4* [MIM 600133]), located near the disease interval, could also be considered as candidate genes. We therefore screened for mutation the entire coding sequence and promotor region of *PLN* (Z99496) and *ME1* (NM002395) genes by PCR and SSCP. We did not detect any gene defects that could cause DCM. *LAMA4* gene has been excluded by linkage analysis using the microsatellite marker D6S416 in intron 29 (X91171) (Dib et al. 1996). The screening of the remaining candidate genes within the 6q12-16 region is in progress in our laboratory.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank: http://www.ncbi.nlm.nih.gov/Genbank/ (for cardiac actin gene [accession numbers J00070, J00071, J00072, and J00073], desmin gene [accession number M58168], *LMNA* gene [accession numbers L12399, L12400, and L12401], *ME1* gene [accession number NM002395], *PLN* gene [accession number Z99496], and *LAMA4* gene [accession number X91171])

Généthon, http://www.genethon.fr (microsatellite markers and chromosome 6 linkage map)

Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/Omim/ (for *CMD1A* [MIM 115200], *CMD1B* [MIM 600884], *CMD1C* [MIM 601493], *CMD1D* [MIM 601494], *CMD1E* [MIM 601154], *CMD1F* [MIM 602067], *CMD1G* [MIM 604145], *CMD1H* [MIM 604288], *COL9A1* [MIM 120210], *MYO6* [MIM 600970], *VEGF* [MIM 192240], *PLN* [MIM 600133], *ME1* [MIM 154250], and *LAMA4* [MIM 600133])

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