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Autosomal Dominant Marfan-like Connective-Tissue Disorder with Aortic Dilation and Skeletal Anomalies Not Linked to the Fibrillin Genes

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Summary

We describe a large family with a connective-tissue disorder that exhibits some of the skeletal and cardiovascular features seen in Marfan syndrome. However, none of the 19 affected individuals displayed ocular abnormalities and therefore did not comply with recognized criteria for this disease. These patients could alternatively be diagnosed as MASS (mitral valve, aorta, skeleton, and skin) phenotype patients or represent a distinct clinical entity, i.e., a new autosomal dominant connective-tissue disorder. The fibrillin genes located on chromosomes 15 and 5 are clearly involved in the classic form of Marfan syndrome and a clinically related disorder (congenital contractural arachnodactyly), respectively. To test whether one of these genes was also implicated in this French family, we performed genetic analyses. Blood samples were obtained for 56 family members, and four polymorphic fibrillin gene markers, located on chromosomes 15 (Fib15) and 5 (Fib5), respectively, were tested. Linkage between the disease allele and the markers of these two genes was excluded with lod scores of −11.39 (for Fib15) and −13.34 (for Fib5), at $\theta = .001$, indicating that the mutation is at a different locus. This phenotype thus represents a new connective-tissue disorder, overlapping but different from classic Marfan syndrome.

Introduction

A number of conditions, such as homocystinuria, annuloaortic ectasia, congenital contractural arachnodactyly (CCA), or familial forms of ectopia lentis and pupillae, share features with the Marfan syndrome (MFS) but are clearly distinct disorders because they display differences in their mode of inheritance and biochemical defects (for review, see Pyeritz 1990). However, in the absence of syndromic boundaries, clear diagnostic criteria, and biochemical or DNA markers, other conditions displaying many manifestations of the syndrome have been reported and coined "formes frustes" (Read et al. 1965; Emmanual et al. 1977). Glesby and Pyeritz (1989) addressed the possibility that they represented other pleiotropic syndromes that constituted a phenotypic continuum with several of the classic heritable disorders of connective tissue, notably MFS. In the case of patients presenting with mitral valve prolapse (MVP), aortic dilation, associated with the skin and skeletal manifestations of a systemic connective-tissue defect, these authors suggested the use of the acronym "MASS [mitral valve, aorta, skeleton, and skin] phenotype." It is still unclear whether this phenotypic continuum results only from mutations in a single gene.

MFS is a dominantly inherited disorder of connective tissue. The syndrome has an estimated prevalence of 4–6/100,000 individuals (Pyeritz and McKusick 1979). This rate may be greatly underestimated, in the absence of a specific diagnostic test, because of great

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phenotypic variability. The cardinal features of the syndrome occur in three systems: skeletal, ocular, and cardiovascular (McKusick 1972). Diagnosis is clinical and relies on established criteria: at least two—and, preferably, three—of the systems involved should show typical abnormalities (Beighton et al. 1988). Clinical features of MFS suggest that an important component of connective tissue could be primarily affected. However, several authors demonstrated by the candidate-gene approach that most of the procollagen genes, as well as the elastin and fibronectin genes, were not genetically linked to the disorder in several families and therefore were not involved in its pathogenesis (for review, see McKusick 1990). At the same time, a new ubiquitous constituent of the extracellular matrix was identified: fibrillin (Sakai et al. 1986). It was implicated in the pathogenesis of MFS, since qualitative and quantitative abnormalities in the staining patterns of microfibrillar fibers were observed in skin and cultured fibroblasts of patients by indirect immunofluorescence studies using antibodies specific for fibrillin (Hollister et al. 1990). Two different experimental approaches were then undertaken: the first used genetic linkage with random probes, in an effort to map the disease locus (Blanton et al. 1990); the second was aimed at isolating and mapping the fibrillin gene. Recently these efforts converged: Kainulainen et al. (1990) first demonstrated linkage between the syndrome and two markers located on the long arm of chromosome 15. Finer mapping in that area located the disease locus (MFS1) between markers D15S1 and D15S48 in 15q15-q21 (Dietz et al. 1991*a;* Sarfarazi et al. 1992). Subsequently and independently, Lee et al. (1991) and Maslen et al. (1991) cloned and partially sequenced a fibrillin gene (Fib15) located in the same chromosomal region. With the polymorphisms detected by the fibrillin cDNA, tight linkage between MFS and the gene was observed in six families in which affected members displayed classic and complete forms of the disorder (Dietz et al. 1991*b;* Lee et al. 1991). Finally, analysis of fibrillin cDNA from patients allowed identification of several missense mutations that implicated fibrillin as the defective protein in some MFS cases, and it therefore demonstrated that Fib15 and MFS1 were the same locus (Dietz et al. 1991*b,* 1992; Kainulainen et al. 1992). In the group of genomic clones that they isolated, Lee et al. (1991) identified two other fibrillin genes, located on chromosomes 5 (Fib5) and 17. The Fib5 gene is also polymorphic and has been shown to be tightly linked to CCA (Lee et al. 1991). Furthermore, Tsipouras et al. (1992) showed that autosomal dominant ectopia lentis was also linked to the Fib15 gene. These reports demonstrate that mutations in the fibrillin genes are involved not only in MFS but also in related disorders that display overlapping skeletal and ocular features.

We have been investigating a large family of more than 170 subjects presenting with an autosomal dominant phenotype with skeletal and cardiovascular abnormalities similar to those of MFS or MASS but without dislocation or subluxation of the lens. The candidate gene, as well as the exclusion mapping approaches, excluded linkage to any of the markers tested (Boileau et al. 1990*a,* 1990*b*). Here we report the results of the linkage analyses performed between the phenotype segregating in this family and polymorphic markers of the Fib15 and Fib5 genes. Our results show that the two fibrillin genes are not linked to this phenotype.

Material and Methods

Clinical Data

Family investigation was undertaken in 1977 after the death of a 39-year-old male (III-32) from aortic dissection. Necropsy reported dilation of ascending aorta at the level of Valsalva sinuses. Histology showed mucoid degeneration of the ascending aorta, with disorganization of the aortic elastic structures. A family pedigree was constructed (a simplified version of the pedigree is shown in fig. 1) (Boileau et al. 1990*a*). Height was compared with values obtained in the French population and was considered abnormal if greater than the mean plus 2 SD calculated for matched age and sex (Sempé et al. 1979). Arm span was considered abnormal if arm-span-over-height ratio exceeded 1.03 (Glesby and Pyeritz 1989). Upper-to-lower-segment-length ratio was compared with the mean minus 2 SD as reported by McKusick (1972); as no normal values have been published for ages over 18 years, we assumed that the ratio did not change later in life. Anterior chest deformity, kyphoscoliosis, arched palate, and arachnodactyly were looked for. Ophthalmological investigations (including slit-lamp examination) were performed to establish myopia, retinal detachment, and ectopia lentis. Echocardiographic examination was performed, using M-mode (Sahn et al. 1978) and two-dimensional imaging to assess aortic diameter at the level of the sinuses of Valsalva and aortic annulus, respectively. All the values were standardized by using body-surface area (BSA), in agreement with Pyeritz and McKusick (1979). Aortic dilation was considered when two-dimensional targeted M-mode aortic diameter was greater than the mean plus 2 SD of published normal

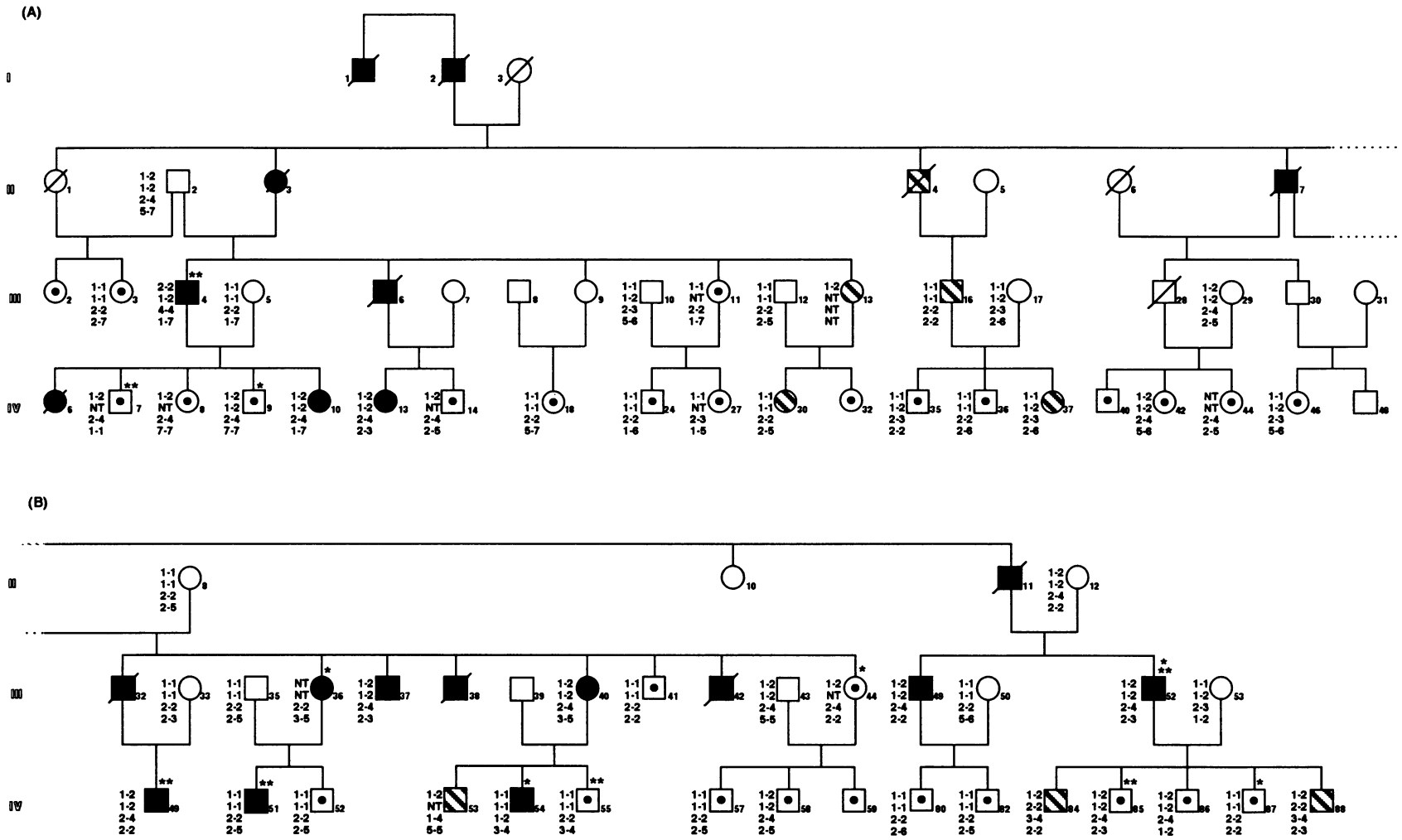

Figure 1 Segregation of Fib15 and Fib5 markers in the French Marfan-like kindred (note that panel *A* and panel *B* represent different parts of a single pedigree; i.e., panel *B* is the rightward extension of panel *A*). Genotypes for each member are shown in the following order (from top to bottom): Fib15/*Bam*HI (alleles 1 and 2), Fib15/*Taq*I (alleles 1 and 2), Fib15/VNTR (alleles 1–4), and Fib5/VNDR (variable number of dinucleotide repeats) (alleles 1–7). Blackened symbols denote affected members, unblackened symbols denote unaffected spouses or unexamined family members, unblackened symbols with a dot in the center denote members considered unaffected, and hatched symbols denote members having unknown status. A slash denotes that the family member is deceased. One or two asterisks indicate subjects demonstrating recombinational events between the family phenotype and, respectively, Fib15 or Fib5 polymorphic markers.

values standardized for age and BSA (Henry et al. 1980). Two-dimensional measurement of the aortic annulus was compared with published data (El Habbal and Sommerville 1989). MVP was diagnosed either if a 3-mm posterior motion of leaflet interfaces behind the C-D line during end-systole could be recorded on M-mode echocardiogram (Devereux et al. 1987) or if, on two-dimensional echocardiogram, the leaflet cooptation occurred on the atrial side on a parasternal long-axis view (Levine et al. 1988). Finally, histology was performed using standard techniques, when an aortic-wall specimen was available (two autopsies and one tissue sample were obtained during surgery).

DNA Marker Analysis

Blood samples were taken from 56 family members, and DNA was isolated according to a method described elsewhere (Henry et al. 1984). Ten micrograms of genomic DNA was digested with the appropriate restriction enzyme according to the manufacturer's instructions (Promega, Madison) and electrophoresed on 0.8% agarose gels. Transfers were done onto Hybond-N membranes (Amersham) by using the Southern blotting procedure with slight modifications (Henry et al. 1984). Prehybridization and hybridization were carried out as recommended (Amersham). Probes were radiolabeled by the random-primer method of Feinberg and Vogelstein (1983), utilizing $\alpha(^{32}P)$dCTP according to standard procedures (Boehringer Mannheim). Autoradiography was obtained on Kodak X-OMAT films at −80°C. Autoradiographic bands were independently scored by two of us. *Bam*HI and *Taq*I digests were hybridized with probe pCML8 to detect two Fib15 intragenic RFLPs (Clark et al. 1991; Dietz et al. 1991*b*).

The $(GT)_n$ and the $(TAAAA)_n$ polymorphic sequences of the Fib5 and Fib15 genes, respectively (Lee et al. 1991), were also tested. Standard PCRs were carried out in a 50-μl volume containing 200 ng of genomic DNA template, 10 pmol of each oligodeoxynucleotide primer, 200 μM each of dGTP, dCTP, dTTP, and dATP, 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, and 2.5 units of *Taq* polymerase (Perkin Elmer Cetus, Norwalk, CT). Samples were processed through 30 cycles consisting of denaturation, annealing, and elongation. PCR conditions and primer sequences used were as reported elsewhere (Lee et al. 1991). Aliquots of the amplified DNAs were electrophoresed on standard denaturing polyacrylamide DNA sequencing gels. DNA was subsequently transferred to Hybond-N+ membranes (Amersham). Hybridization was performed with one of the primers used in the amplification step, labeled at its 3′ end with $\alpha(^{32}P)$dCTP by the terminal transferase (Boehringer Mannheim) according to the method of Hazan et al. (1992). Autoradiography was carried out after two washes in 2 × SSC and 0.1 % SDS.

Linkage Analysis

The MLINK subprogram of the LINKAGE software package (Lathrop et al. 1984) was used for pairwise and multipoint linkage analyses. Allele frequencies of DNA markers previously published were used in the analyses. When previously published data were unavailable, these frequencies were calculated from the study of unrelated members of the family. Assumptions applied to the linkage analyses were an autosomal dominant mode of inheritance, a frequency for the disease allele of .00002, and a penetrance of 1.0, since there was no evidence of skipped generations in the pedigree.

Results

Clinical Data

Clinical findings in family members are summarized in table 1. Positive diagnosis of affected individuals relied solely on skeletal and cardiovascular features, as no significant ophthalmological anomaly was observed. We therefore simply considered involvement of a connective-tissue disorder in establishing the status. In the absence of diagnosis criteria for the family phenotype, patient status was established independently by two of us (G.J. and O.D.), with regard to the actual clinical follow-up. Only family members who showed major manifestations in at least one of the systems (skeletal or cardiovascular) and were first-degree relatives of an affected member were considered as definitely affected. Conversely, family members with no abnormality in any of the systems and a few subjects who presented with isolated minor skeletal or cardiovascular findings were considered unaffected.

Nineteen individuals were considered "affected" (II-3, II-7, II-11, III-4, III-6, III-32, III-36, III-37, III-38, III-40, III-42, III-49, III-52, IV-6, IV-10, IV-13, IV-49, IV-51, and IV-54). III-38, who died of an acute aortic dissection, disclosed the complete family phenotype: necropsy showed skeletal abnormalities (height 186 cm, arachnodactyly, pectus excavatum), a past history of surgery for inguinal hernia, and dilation of the ascending aorta. Histological examination of the ascending aorta revealed discontinuation of elastic lamellae and increased deposits of mucopolysaccharide-like ma-

Table 1

Individual Clinical Features

Patient[a] (age in years, sex)	Anthropometrics			Skeletal Features						Cardiovascular Features				
												Aortic		
	BSA (m^2)	Height (cm)	Arm Span (cm)	UL/LL[b]	SC[c]	Palate[d]	AR[e]	Pectus[f]	Back[g]	MVP	MR	Root Diameter[h] (mm)	TM[i] (mm)	Dilation[j] (SD)
III-2 (45, F)	1.85	170	166	1.00	0	0	0	0	0	0	0	20	33	1.24
III-3 (43, F)	1.68	170	167	1.00	0	0	0	0	0	0	0	20	27	−.60
III-4 (56, M)	2.27	188	198	.98	0	1	0	0	0	0	0	25	43	3.45
III-11 (51, F)	1.94	170	175	1.05	0	0	0	0	0	0	0	20	30	−.26
III-13 (49, F)	1.79	180	172	.80	0	0	0	0	0	0	0	21	31	.47
III-16 (47, M)	2.21	185	...	...	...	...	...	...	...	0	...	21	36	1.52
III-36 (48, F)	1.36	167	180	.92	0	0	1	0	0	0	0	21	36	3.65
III-37 (48, M)	2.18	190	196	.90	0	0	0	0	0	0	0	25	41	3.29
III-40 (40, F)	1.70	172	169	.89	0	0	1	1	0	1	1	25	35	2.62
III-41 (43, M)	2.08	185	185	1.18	0	0	0	0	0	0	0	22	32	.50
III-44 (37, F)	1.63	170	165	.81	0	0	0	0	0	0	0	19	27	−.27
III-52 (43, M)	2.09	185	182	1.18	0	0	0	0	0	0	0	22	39	3.01
IV-7 (24, M)	2.03	184	197	1.02	0	0	0	0	0	0	0	22	31	.98
IV-8 (22, F)	1.76	176	186	.73	0	1	0	0	0	0	0	19	27	.05
IV-9 (17, M)	1.95	193	193	.97	1	1	0	0	0	0	0	25	28	.26
IV-10 (12, F)	1.37	160	168	.80	1	1	1	1	0	1	1	20	27	1.64
IV-13 (22, F)	1.62	168	170	.80	0	0	1	0	0	0	0	20	33	2.95
IV-14 (22, M)	2.00	180	183	1.00	0	0	0	0	0	0	0	19	28	−.05
IV-18 (28, F)	1.76	168	173	1.37	0	0	0	0	0	0	0	20	27	−.20
IV-24 (27, M)	2.24	185	190	1.12	0	0	0	0	0	0	0	23	31	.45
IV-27 (13, F)	1.51	160	165	1.20	0	0	0	0	0	0	0	18	28	1.56
IV-30 (38, F)	1.83	173	173	1.11	0	0	0	0	0	0	0	21	39	3.90
IV-32 (12, F)	1.52	173	165	.82	0	0	0	0	0	0	0	17	25	.23
IV-35 (20, M)	2.04	185	190	1.20	0	1	1	0	1	0	0	27	25	−1.23
IV-36 (15, M)	1.34	161	159	1.18	0	1	1	0	0	0	0	15	20	−1.72
IV-37 (9, M)	1.00	140	138	1.16	0	0	1	0	0	1	1	13	24	1.85
IV-40 (27, F)	1.99	180	182	1.00	0	0	0	0	0	0	0	19	30	.56
IV-42 (26, F)	1.43	162	158	.88	0	0	0	0	0	0	0	20	30	2.03
IV-44 (25, F)	1.68	177	183	.97	0	0	0	1	0	0	0	22	31	1.77
IV-46 (31, F)	1.64	167	166	.92	0	0	0	0	0	0	0	20	28	.37
IV-49 (27, M)	2.09	192	200	.90	1	1	1	1	0	0	0	20	40	4.16
IV-51 (17, M)	1.78	182	196	1.12	0	0	1	0	0	0	1	20	35	3.57
IV-52 (15, F)	1.43	162	162	1.00	0	0	0	0	0	0	0	18	24	−.12
IV-53 (16, M)	1.92	185	186	.80	0	0	0	0	0	0	0	21	32	1.98
IV-54 (15, M)	1.71	172	178	1.05	0	0	1	0	0	0	0	22	32	2.62
IV-55 (11, M)	1.40	155	151	1.07	0	0	0	0	0	0	0	20	25	.63
IV-57 (12, M)	1.56	165	170	.94	1	0	0	0	0	0	0	20	23	−.78
IV-58 (10, M)	1.28	159	146	1.01	0	0	0	0	1	0	0	16	22	−.38
IV-59 (9, M)	1.06	137	153	.93	1	0	0	0	0	0	0	14	22	.47
IV-80 (19, M)	2.11	194	194	.88	0	1	0	0	1	0	0	22	31	1.03
IV-82 (15, M)	1.80	184	180	1.04	0	0	0	0	1	0	0	19	25	−.58
IV-84 (16, M)	1.49	171	172	1.16	0	0	0	0	0	0	0	21	30	2.37
IV-85 (14, M)	1.39	164	164	.97	0	0	0	0	0	0	0	17	27	1.45
IV-86 (13, M)	1.21	147	145	.96	0	0	0	0	0	0	0	19	25	1.18
IV-87 (10, M)	1.09	138	133	1.04	0	0	0	0	0	0	0	17	21	−.19
IV-88 (9, M)	1.00	137	128	1.00	0	1	0	0	0	0	0	20	25	2.38

Note.—Deceased family members with incomplete clinical data are described in Results.

[a] No. is as in fig. 1.

[b] Upper-over-lower-segment length ratio.

[c] Scoliosis.

[d] Arched palate.

[e] Arachnodactyly.

[f] Pectus excavatum or recurvatum.

[g] Straight back.

[h] Measured by using two-dimensional echocardiography.

[i] Aortic diameter, measured by using M-mode echocardiography.

[j] M-mode aortic diameter, expressed as the number of SD above (+) or below (−) the mean normal value reported by Henry et al. (1980).

terial in the media. Some first-degree relatives presented both aortic dilation and skeletal abnormalities: III-36 and her son IV-51, III-37, and III-40, who also disclosed MVP and mitral regurgitation (MR). IV-49 disclosed skeletal features and aortic dilation, and his father (III-32) died suddenly at the age of 35 years. Sudden death was reported in three other subjects: II-7 (mesenteric infarction at age 57 years); III-32, the propositus (aortic dissection at age 39 years); and III-42 (aortic dissection at age 29 years). IV-10 presented all the skeletal abnormalities that were looked for. She also disclosed MVP with regurgitation. Her sister (IV-6) died suddenly, after complaining of chest pain, at age 9 years and disclosed joint hyperlaxity. Her father (III-4) disclosed aortic dilation and skeletal features. Her father's mother (II-3) died suddenly at the age of 35 years, presumably from aortic rupture. IV-13 presented aortic dilation and skeletal abnormalities. Her father (III-6) died in an accident and has not been studied. Because her father's brother presented all the typical features of the disease, we considered IV-13 as affected. She is the only affected individual who presented with myopia. III-49 underwent a Bentall procedure because of aortic dissection, but no anthropomorphic measurements could be obtained. His father (II-11) died suddenly at age 35 years. III-52, III-49's brother, presented aortic dilation without any objective skeletal anomaly. These two patients, despite lack of evidence for skeletal involvement, have been considered as affected, because of major aortic involvement responsible for one dissection and, probably, for the father's death. IV-54 presented with aortic dilation and minor skeletal abnormality at age 15 years; because his mother (III-40) presented all the typical features (cardiovascular and skeletal), we considered mother and son as affected.

No definite diagnosis could be made for six other patients: IV-30 showed aortic dilation and no skeletal anomaly, in contrast to her mother (III-13), who disclosed minor skeletal anomalies and no cardiac involvement; IV-37 displayed, at age 9 years, minor skeletal abnormalities, as well as MVP with MR; IV-53, who is tall, has a borderline aortic dilation (+2 SD above the mean); IV-84 (at age 16 years) and IV-88 (at age 9 years) disclosed isolated borderline aortic dilation. These patients were scored as "unknown" in the genetic analyses, in order to avoid spurious results due to misclassification. Subjects II-4 and III-16, who are grandfather and father, respectively, of subject IV-37, and who thus could be asymptomatic carriers, were also included in this group.

Finally, we considered as unaffected 12 patients (III-44, IV-7, IV-8, IV-9, IV-32, IV-35, IV-36, IV-44, IV-57, IV-58, IV-59, and IV-80) who presented isolated minor skeletal anomalies without cardiovascular involvement and 17 patients (III-2, III-3, III-11, III-41, IV-14, IV-18, IV-24, IV-27, IV-40, IV-42, IV-46, IV-52, IV-55, IV-82, IV-85, IV-86, and IV-87) who presented no anomaly in any of the systems.

Linkage Analysis

The family was tested for genetic linkage to three polymorphic markers located within the Fib15 gene, by using the candidate-gene approach. Since many individuals in the family were not available for genotyping, most meioses are phase unknown. The construction of fibrillin haplotypes is impossible, and only possible recombinants can be identified. Six recombinants were observed (fig. 1): three affected (III-36, III-52, and IV-54) individuals and three (III-44, IV-9, and IV-87) unaffected individuals (table 1). In effect, while allele 4 of the Fib15 VNTR segregates with the phenotype in all other individuals, the genotype of III-36 (2/2) excludes linkage of allele 4 with the disease. Furthermore, allele 4 segregates with a normal phenotype in subjects III-44 and IV-9. No double intragenic recombinants were found. Therefore, linkage was excluded between the disease and the Fib15 markers (table 2).

Since the MFS-like phenotype observed in this family is not linked to the Fib15 gene but could be due to a defect in another of the fibrillin genes, we investigated linkage with the VNDR within the Fib5 locus. Again, seven recombinants were observed (fig. 1). Strongly negative lod scores were obtained, and linkage was excluded over an area of more than 4 cM on either side of the gene (table 2).

Discussion

We report here a clinical and genetic study of a large family, affected by a dominant autosomal disease, that comprises more than 170 subjects, 56 of whom have been studied. Clinical features associated cardiovascular anomalies (i.e., aortic dilation in 11 members and MVP in 3, producing aortic dissection or rupture in 7 patients, 6 of whom died suddenly) and skeletal anomalies (great height, great arm span, arachnodactyly, skoliosis, and pectus). No specific ocular anomaly could be found despite systematic ophthalmological examination including split-lamp examination. Joint laxity was not systematically looked for but was reported in several members of this family. Although these features

Table 2

Two-Point Lod Scores between Family Phenotype and Fib15 and Fib5 Polymorphic Markers

	Z AT θ =						
LOCUS	.001	.01	.05	.1	.2	.3	.4
Fib15 haplotype	−11.39	−5.47	−1.63	−.30	.51	.51	.22
Fib5	−13.34	−6.43	−1.92	−.31	.72	.77	.41

were evocative of MFS, this diagnosis could not be made by using recognized criteria (Beighton et al. 1988), since a patient who had been operated on for aortic dissection would have been considered as unaffected. Association of skeletal and cardiovascular features excludes MVP syndrome, annuloaortic ectasia, CCA, and Stickler syndrome. The MASS phenotype (Glesby and Pyeritz 1989) may be considered in this family. However, since MVP and skin involvement are only documented in a few of the affected family members, this diagnosis seems unlikely. The only remaining hypothesis is that this phenotype represents a new connective-tissue disorder overlapping but different from classic MFS.

Linkage analysis excluded the fibrillin gene located on chromosome 15. This exclusion is based on the identification of several recombinants that could have been wrongly scored because of any one of three factors: nonpaternity, misclassification of individuals, and intragenic recombinational events. In our family, nonpaternity cannot be directly addressed, since all the sibs of generation II are deceased, and blood samples could not be obtained for several generation III parents. However, over 60 polymorphic markers have now been tested, among which are numerous highly informative VNTRs, and nonpaternity can be indirectly ruled out, since no incompatible phase has been detected (Boileau et al. 1990*a*, 1990*b*). To avoid misclassification of some family members for whom diagnosis could not be ascertained in the absence of clearly defined criteria for this syndrome, eight individuals were scored as unknown in the genetic analyses. Furthermore, if these individuals are scored as affected, then further recombinants are identified. Conversely, among the six possible recombinants, phenotypic misclassification is ruled out, since all are now well past the age of 20 years and include three undoubtedly affected subjects and three who display no sign of the disease. The last factor that may affect the scoring of recombinants is the existence of recombinational events occurring within the fibrillin gene. However, the maximum likelihood of linkage between the family phenotype and the Fib15 markers was at $\theta = .20$ ($Z = 0.51$). Furthermore, since, when nine anonymous chromosome 15 markers that flank the Fib15 gene were used, linkage was excluded in an area of 83 cM around this locus (data not shown), the last hypothesis is extremely unlikely. Therefore, the lack of linkage is not due to analytical differences between the family and the MFS families reported as strongly linked to the Fib15 locus. Thus, our results demonstrate that the two diseases are not allelic.

The molecular basis of this phenotype is unknown. It could be due either to alterations of another member of the fibrillin protein family or, alternatively, to anomalies in proteins necessary for the assembly of fibrillin-rich microfibrils. Cloning of the Fib15 gene has led to the discovery of a gene family in which two members, located on chromosomes 5 and 17, have been identified (Lee et al. 1991). Since polymorphic markers have been published only for the Fib5 gene, linkage analyses could only be performed for this locus. Again linkage was excluded, which is not unexpected when the differences between the family disease and CCA are considered. Until new candidate genes are available, mapping of the disease locus will rely on the reverse-genetic approach. In conclusion, we have shown that the connective-tissue disorder that segregates in this family as an autosomal dominant disease and that is characterized by the association of skeletal and cardiovascular features is an entity distinct from classic MFS, since it is not associated with a fibrillin gene defect.

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References

Beighton P, DePaepe A, Danks D, Finidori G, Gedde-Dahl T, Goodman R, Hall JG, et al (1988) International nosology of heritable disorders of connective tissue, Berlin, 1986. Am J Med Genet 29:581–594

Blanton SH, Sarfarazi M, Eiberg H, deGroot J, Farndon PA, Child AH, Pope FM, et al (1990) An exclusion map of Marfan syndrome. J Med Genet 27:73–77

Boileau C, Jondeau G, Bonaïti-Pellié C, Coulon M, Delorme G, Dubourg O, Bourdarias JP, et al (1990*a*) Linkage analysis of five fibrillar collagen loci in a large French Marfan syndrome family. J Med Genet 27:78–81

Boileau C, Jondeau G, Coulon M, Delorme G, Dubourg O, Bonaïti C, Bourdarias JP, et al (1990*b*) Linkage studies for Marfan syndrome and for markers on chromosomes 13 and 15. Am J Hum Genet 47:A682

Clark BA, Maslen CL, Sakai LY, Dhalimi MA, Litt R, Litt M (1991) A BamHI polymorphism at the fibrillin (FNB) locus. Nucleic Acids Res 19:4309

Devereux RB, Kramer-Fox R, Shear K, Kligfield P, Pini R, Savage DD (1987) Clinical and epidemiological issues in mitral valve prolapse—diagnosis and classification of severity of mitral valve prolapse: methodologic, biologic, and prognostic considerations. Am Heart J 113:1265–1280

Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, Puffenberger EG, et al (1991*a*) Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature 352:337–339

Dietz HC, Pyeritz RE, Hall BD, Cadle RG, Hamosh A, Schwartz J, Meyers DA, et al (1991*b*) The Marfan syndrome locus: confirmation of assignment to chromosome 15 and identification of tightly linked markers at 15q15-q21.3. Genomics 9:355–361

Dietz HC, Pyeritz RE, Puffenberger EG, Kendzior RJ, Corson GM, Maslen CL, Sakai LY, et al (1992) Marfan phenotype variability in a family segregating a missense mutation in the epidermal growth factor-like motif of the fibrillin gene. J Clin Invest 89:1674–1680

El Habbal M, Sommerville J (1989) Size of the normal aortic root in normal subjects and in those with left ventricular outflow obstruction. Am J Cardiol 63:322–326

Emmanual R, Marcomichelakis J, Moores EC, Jefferson KE, Macfaul PA, Withers R (1977) Formes frustes of Marfan's syndrome presenting with severe aortic regurgitation: clinico-genetic study of 18 families. Br Heart J 39:190–197

Feinberg A, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Glesby MJ, Pyeritz RE (1989) Association of mitral valve prolapse and systemic abnormalities of connective tissue: a phenotypic continuum. JAMA 262:523–528

Hazan J, Dubay C, Pankowiak AP, Becuwe N, Weissenbach J (1992) A genetic linkage map of human chromosome 20 composed entirely of microsatellite markers. Genomics 12:183–189

Henry WL, Gardin JM, Ware JH (1980) Echocardiographic measurements in normal subjects from infancy to old age. Circulation 62:1054–1061

Henry I, Uzan G, Nicolas H, Weil D, Nicolas H, Kaplan JC, Marguerie C, et al (1984) The genes coding for Aα-, Bβ-, and γ-chains of fibrinogen map to 4q2. Am J Hum Genet 36:760–768

Hollister DW, Godfrey M, Sakai LY, Pyeritz RE (1990) Immunohistologic abnormalities of the microfibrillar-fiber system in the Marfan syndrome. N Engl J Med 323:152–159

Kainulainen K, Pulkkinen L, Savolainen A, Kaitila I, Peltonen L (1990) Location on chromosome 15 of the gene defect causing Marfan syndrome. N Engl J Med 323:935–939

Kainulainen K, Sakai L, Child A, Pope M, Puhakka L, Ryhänen L, Palotie A, et al (1992) Two mutations in Marfan syndrome resulting in truncated fibrillin polypeptides. Proc Natl Acad Sci USA 89:5917–5921

Lathrop GM, Lalouel J-M, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Lee B, Godfrey M, Vitale E, Hori H, Mattei MG, Sarfarazi M, Tsipouras P, et al (1991) Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. Nature 352:330–334

Levine RA, Stathogiannis E, Newell JB, Harrigan P, Weyman AE (1988) Reconsideration of echocardiographic standards for mitral valve prolapse: lack of association between leaflet displacement isolated on the four-chamber view and independent echocardiographic evidence of abnormality. J Am Coll Cardiol 11:1010–1019

McKusick VA (1972) Heritable disorders of connective tissue, 4th ed. Mosby, St Louis, pp 61–137

——— (1990) Mendelian inheritance in man. Johns Hopkins University Press, Baltimore

Maslen CL, Corson GM, Maddox BK, Glanville RW, Sakai LY (1991) Partial sequence of a candidate gene for the Marfan syndrome. Nature 352:334–337

Pyeritz RE (1990) Marfan syndrome. In: Emery EH, Rimoin DL (eds) Principle and practice of medical genetics, 2d ed. Churchill Livingstone, New York, pp 1047–1063

Pyeritz RE, McKusick VA (1979) The Marfan syndrome: diagnosis and management. N Engl J Med 300:772–777

Read RC, Thal AP, Wendt VE (1965) Symptomatic valvular myxomatous transformation (the floppy valve syndrome): a possible forme fruste of the Marfan syndrome. Circulation 32:897–910

Sahn DJ, DeMaria A, Kisslo J, Weyman A (1978) The committee on M-mode standardization of the American Society of Echocardiography: recommendations regarding

quantification in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 58:1072–1083

Sakai LY, Keene DR, Engvall E (1986) Fibrillin, a new 350-kD glycoprotein, is a component of extracellular microfibrils. J Cell Biol 103:2499–2509

Sarfarazi M, Tsipouras P, Del Mastro R, Kilpatrick M, Farndon P, Boxer M, Bridges A, et al (1992) A linkage map of 10 loci flanking the Marfan syndrome locus on 15q: results of an international consortium study. J Med Genet 29:75–80

Sempé M, Pédron G, Roy-Pernot MP (1979) Auxologie—méthodes et séquences. Theraplix, Paris

Tsipouras P, Del Mastro R, Sarfarazi M, Lee B, Vitale E, Child AH, Godfrey M, et al (1992) Genetic linkage of the Marfan syndrome, ectopia lentis, and congenital contractural arachnodactyly to the fibrillin genes on chromosomes 15 and 5. N Engl J Med 326:905–909