Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 4486–4488, May 1991
Medical Sciences

Marfan syndrome is closely linked to a marker on chromosome 15q1.5→q2.1

(genetic map/connective tissue disorder/restriction fragment length polymorphism/genetics)

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Communicated by Mary Jane Osborn, February 22, 1991

ABSTRACT **Marfan syndrome is a systemic disorder of the connective tissue inherited as an autosomal dominant trait. The disorder imparts significant morbidity and mortality. The etiology of the disorder remains elusive. A recent study localized the gene for Marfan syndrome on chromosome 15. We present data showing that marker *D15S48* is genetically linked to Marfan syndrome. Pairwise linkage analysis gave a maximum lod (logarithm of odds) score of $Z = 11.78$ at $\theta = 0.02$. Furthermore our data suggest that the Marfan syndrome locus is possibly flanked on either side by *D15S48* and *D15S49*.**

Marfan syndrome is an autosomal dominant disorder of the connective tissue, characterized by cardiovascular, musculoskeletal, ocular, and pulmonary abnormalities (1). This condition is estimated to have a prevalence of 4–6 per 100,000 individuals, imparts significant morbidity, and if untreated, reduces significantly the life expectancy (2). The diagnosis of the condition is clinical. To date there is no diagnostic test specific for the condition because the underlying genetic basis of Marfan syndrome (MFS1) has eluded definition despite numerous histological, ultrastructural, and biochemical investigations of various candidate molecules found in the extracellular matrix of the connective tissue (3–10). Positional mapping by linkage studies to markers of known chromosomal localization has been used to map and subsequently clone the genes in several genetic disorders, among them, Duchenne muscular dystrophy, cystic fibrosis, and neurofibromatosis. Kainulainen *et al.* (11) demonstrated genetic linkage of the *MFS1* gene to two anonymous DNA markers mapped on chromosome 15. We report here on the results of our studies in families with MFS1, using five markers located on chromosome 15. Our findings confirmed the mapping of the *MFS1* gene on this chromosome although in a more proximal location to the one originally reported. Our data suggest that two of the markers are possibly flanking the *MFS1* gene.

MATERIALS AND METHODS

Pedigrees. Twelve three-generation families with MFS1 were ascertained in which the proband presented with cardiovascular, musculoskeletal, and ocular involvement. The number of potential meioses of the family panel was 147, of which 71 were of known meiosis phase. One of us (P.T.) personally examined the great majority of individuals included in this study. The clinical evaluation included anthropometric measurements, clinical examination, a dilated slit lamp exami-nation by an ophthalmologist, and an echocardiogram (4). The composite clinical phenotype of each family studied was consistent with the diagnosis of MFS1 according to diagnostic criteria previously established and widely accepted (12). In one of the families, the extent of cardiovascular manifestations was mitral valve prolapse. In a second family, a mild subluxation of the crystalline lens was observed. In a third family, no ocular manifestations were noted.

Restriction Fragment Length Polymorphism (RFLP) Genotyping. High molecular weight DNA was extracted from peripheral blood. Five micrograms of DNA was digested with the appropriate restriction enzymes, fractionated on 0.8–1% agarose gels, transferred to nylon membranes, and then hybridized to DNA probes labeled with ^{32}P by either nick-translation or the random-primer technique as described elsewhere (13, 14). Before genotyping the families for any of the polymorphic markers, DNA from all subjects was evaluated to verify the stated familial relationships by using probes for at least two of the highly polymorphic loci that have been reported (15).

Marker Loci. Five polymorphic marker loci on chromosome 15 were used for analysis of linkage with *MFS1* (16). The marker loci included *D15S48* (CRI-L442), *D15S49* (CRI-L1204), *D15S51* (CRI-P452), *D15S45* (pEFZ33), and *D15S29* (pEFD49.3). The *D15S48* probe identifies two *Msp* I dimorphic allele systems that can be haplotyped; both systems were used in this study. The *D15S49* and *D15S51* loci identify *Msp* I dimorphisms, while *D15S45* identifies a *Hin*dIII dimorphism. Finally, *D15S29* identifies two dimorphic allele systems generated by *Msp* I and *Taq* I, of which only one (*Taq* I) was used in this study.

Linkage Analysis. Pairwise linkage analysis between *MFS1* and each marker was performed by using the personal computer-adapted program LIPED (17) and the MLINK program from the LINKAGE package (18). The Marfan locus was defined as having full penetrance. Individuals less than 5 years old were excluded from the analysis. Likelihoods were calculated for recombination fractions from 0 to 0.5 and expressed as lod (logarithm of odds) scores. Sex average and male and female lod scores for various recombination fractions were obtained for each marker. Confidence intervals were constructed by subtracting one from the maximum lod score (19). Multipoint linkage analysis was performed by using the program CRIMAP (20).

RESULTS

Four of the five markers used gave informative results in at least five families. Two of the markers, *D15S45* and *D15S29*, were previously reported by Kainulainen *et al.* (11) to be tightly linked to *MFS1*. The other three markers, *D15S48*,

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Abbreviations: MFS1, Marfan syndrome; RFLP, restriction fragment length polymorphism.

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D15S49, and *D15S51*, were chosen for their map position deemed to be proximal (centromeric) to the previous two. None of the five markers was linked to *MFS1* at $\theta = 0.0$, where θ is the recombination fraction (Table 1). The marker *D15S48* was found to be the nearest to the *MFS1* locus, and pairwise linkage analysis gave a maximum lod score of $Z = 11.78$ at $\theta = 0.02$. The results of the pairwise analysis between each marker used in the study and *MFS1* are presented in Table 1. In contrast to what has been previously reported, our analysis gave maximum lod scores of $Z = 2.92$ at $\theta = 0.12$ and $Z = 0.77$ at $\theta = 0.27$ between *D15S45*, *D15S29*, and MFS1, respectively. Of the other two markers, *D15S49* and *D15S51*, the former gave a maximum $Z = 2.16$ at $\theta = 0.17$, while the latter was essentially not informative in any of the families tested, giving a maximum lod score of $Z = 0.31$ at $\theta = 0.0$. *D15S51* was not used any further in the multipoint linkage analysis. We reviewed the two published genetic maps of chromosome 15 (21, 22) and the CEPH (Center for the Study of Human Polymorphism) data base (23) to ascertain the most likely order of the four markers. The most likely order was determined to be: *D15S48*···*D15S49*···*D15S45*···*D15S45*. The likelihood ratio of the previous order to the next most likely one was estimated to be 10^6:1 (P.T. and M.S., unpublished data). We then fixed the genetic distances between the four markers (Fig. 1) and attempted by multipoint linkage analysis to identify the most likely position of the *MFS1* locus within that frame. In Fig. 1, the two almost equal likely orders are shown; the *MFS1* locus is located either proximal to *D15S48* or between *D15S48* and *D15S49*.

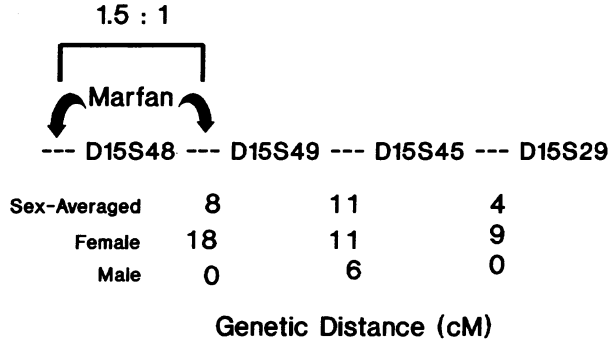

FIG. 1. Multipoint map of the *MFS1* locus region.

DISCUSSION

The clinical phenotype of MFS1 was originally described almost a century ago, and since then it has been expanded continuously (1). MFS1 is the prototypical heritable disorder of the connective tissue with manifestations from various systems and significant variability of expression among affected individuals. The Mendelian nature of the disorder is well established.

Numerous investigations aiming to unravel the etiology of MFS1 have been unsuccessful. It has been reasoned that the mutant molecule must be widely distributed in the tissues to effectuate its pleiotropy, a cardinal feature of the disorder. Elastin (3), various types of fibrillar collagens (4), decorin (5), proteoglycans (6), β-glucuronidase (7), and elastase (8) are some of the candidate molecules investigated in relation to MFS1. The role of fibrillin, a 350-kDa cysteine-rich glycoprotein found in the microfibrillar fiber system, has generated considerable interest. The primary structure of fibrillin is not well known with the exception of two small nonoverlapping polypeptides (24). Fibrillin is found in all tissues involved in MFS1 (25), and it is one of a number of structural proteins found in the microfibrills (26).

Immunohistochemical and *in vitro* studies on cultured skin fibroblasts from patients with MFS1 have shown various defects in approximately 70% of the cases studied (9, 10). The abnormal findings can be broadly categorized as defects in the synthesis, secretion, or extracellular assembly of the fibrillin (10). To date no fibrillin-specific cDNA or genomic clones have been reported, and the fibrillin gene localization remains unknown. The definite proof of a causal association between fibrillin and MFS1 will await either the identification of mutations within the fibrillin gene in patients with MFS1 or the establishment of genetic linkage using fibrillin gene-specific markers.

Positional mapping has been the alternative approach used to identify the cause of MFS1. The generation of an exclusion map (27) facilitated the mapping of the *MFS1* locus on chromosome 15. Our findings are in agreement with the conclusion of Kainulainen *et al.* (11) placing the *MFS1* locus on chromosome 15. However, our data do not support their conclusion that *MFS1* is tightly linked to the markers *D15S45* and *D15S29* at $\theta = 0.0$. To the contrary, the *MFS1* gene is located much closer to the marker *D15S48* as a lod score of 11.78 at $\theta = 0.02$ would strongly indicate. It must be noted that the genetic distance between *D15S48* and *D15S45* has been estimated to be 20 centimorgans (cM) approximately (P.T. and M.S., unpublished data). The discrepancy between our findings and those previously reported could be explained on the basis of: (*i*) two *MFS1* loci being present on chromosome 15, (*ii*) genetic distance between *D15S48* and *D15S45* being smaller than the estimated one, and (*iii*) incorrect assumptions being made on the phenotypic status of some genotyped individuals.

We attempted to identify markers flanking the *MFS1* gene. Based on data derived from the integration of the two published genetic maps of chromosome 15 (21, 22), the most likely order of the four markers used in our study is the following; centromere–*D15S48*–*D15S49*–*D15S45*–*D15S29*–telomere. This order was derived from the analysis of the genotypes deposited in the CEPH data base (23). Multipoint linkage analysis of data from our family panel suggests that the *MFS1* locus is located either proximal to *D15S48* or between *D15S48* and *D15S49* (Fig. 1). It is obvious that more

Table 1. Pairwise lod scores between *MFS1* and chromosome 15q markers

Locus	Z at θ value											θ_{max}	Z_{max}
	0.0	0.01	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45		
D15S48	−∞	11.66	11.58	10.72	9.60	8.32	6.93	5.46	3.95	2.47	1.11	0.02*	11.78
D15S49	−∞	−2.41	0.82	1.84	2.14	2.12	1.91	1.59	1.20	0.77	0.35	0.17†	2.16
D15S51	−∞	0.31	0.31	0.30	0.26	0.22	0.18	0.14	0.10	0.06	0.04	0.00‡	0.31
D15S45	−∞	0.11	2.38	2.89	2.87	2.63	2.25	1.79	1.29	0.79	0.35	0.12§	2.92
D15S29	−∞	−8.91	−2.96	−0.80	0.15	0.60	0.76	0.73	0.59	0.37	0.15	0.27¶	0.77

*The −1 lod confidence interval = 0.00–0.10.
†The −1 lod confidence interval = 0.06–0.36.
‡The −1 lod confidence interval = 0.00–0.50.
§The −1 lod confidence interval = 0.04–0.28.
¶The −1 lod confidence interval = 0.12–0.50.

families with MFS1 must be genotyped before the relative location of the *MFS1* locus is confirmed. The question of genetic heterogeneity of MFS1 has not been resolved yet. Our data were insufficient for a definite conclusion to be drawn. The physical location of the *MFS1* locus is 15q1.5 → q2.1. This localization is presumed because *D15S1*, a marker tightly linked to *D15S48* (21, 22) has been localized by *in situ* hybridization on this region (28). It would be a very interesting to know whether the fibrillin locus is also located in the same region.

Markers flanking the *MFS1* locus must be identified before any application is attempted for diagnostic purposes. The presence of tightly linked anchor markers is also an important step before any strategy for the cloning of the *MFS1* gene is undertaken.

This study would not have been possible without the cooperation of affected individuals and their families, to whom the authors express their gratitude. Numerous colleagues from the United States, Canada, and the United Kingdom referred families to us and helped in collecting clinical information. We wish to thank Mr. William Murphy and Ms. Mary Pat O'Connell for expert technical assistance; Dr. Peter O'Connell of the Howard Hughes Medical Institute, Salt Lake City; Dr. Leena Peltonen of the National Public Health Institute, Helsinki, Finland, for providing generous gifts of certain probes; and Dr. Ivan Balasz of Lifecodes Corp. for assisting in DNA identity testing. This project was supported in part by a research grant from the Coles Family Foundation to one of the authors (P.T.).

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