Linkage analysis in Marfan syndrome

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Abstract

We have analysed 40 marker loci on 13 chromosomes for linkage with Marfan syndrome. None of the loci was linked to the Marfan syndrome locus at $\theta=0.00$. This study provides a basis for an exclusion map and for further collaboration in mapping of the locus.

Marfan syndrome (MS) is an autosomal dominant disorder of the connective tissue, characterised by cardiovascular, musculoskeletal, ocular, and pulmonary abnormalities. This condition is estimated to have a prevalence of 4 to 6 per 100 000 persons and imparts significant morbidity and mortality.^{1,2} Although the clinical manifestations of this disorder have been very well described,¹ to date there is only one documented genetic defect in one family. This was originally reported by Byers *et al*³ to be a small polypeptide insertion in the $\alpha 2$ chain of type I collagen, and has recently been shown by Phillips *et al*⁴ to be a single nucleotide change that results in a substitution of glutamine for arginine at position 618 in the triple helical domain of the protein. However, the causal relationship between this finding and the phenotype remains unclear. Despite numerous histological, ultrastructural, and biochemical investigations⁵⁻⁹ and genetic linkage studies using conventional protein and cell surface markers,^{10,11} as well as the candidate gene approach,¹²⁻¹⁷ the underlying genetic basis of MS has eluded definition.

For this reason, we developed the strategy of using restriction fragment length polymorphisms (RFLPs) that are associated with random probes throughout the genome to map the MS locus. We present the results of our analysis of 10 three generation families here.

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Materials and methods

PEDIGREES

Ten three generation families with MS were ascertained, in which the proband had involvement of the cardiovascular, musculoskeletal, and ocular systems. One of us (PT) personally examined 100 affected and unaffected subjects included in this study. The clinical evaluation usually included anthropometric measurements, clinical examination, a dilated slit lamp examination by an ophthalmologist and an echocardiogram. The diagnosis of MS was made if a subject had at least two major manifestations and one minor manifestation, as described in table 1, which summarises the clinical features of this condition. The phenotype in all families was consistent with the above diagnostic criteria. Family 1 lacked aortic dilatation but certain persons had mitral valve prolapse. In family 6 the lenses were only mildly

Table 1 Major and minor manifestations of Marfan syndrome.

System	Major findings	Minor findings
Ocular	Ectopia lentis	Myopia Retinal detachment
Cardiovascular	Aortic regurgitation Aortic root diameter dilatation	Mitral valve prolapse (clinically and on echocardiogram)
Musculoskeletal	Kyphosis Pectus deformity Upper to lower segment ratio at least 2 SD below mean for age	Tall stature Arachnodactyly Joint laxity
Pulmonary		Pneumothorax

Table 2 Structure of MS pedigrees.

Family no	No of affected	No of unaffected	Potential meioses	Phase known meioses
MS1	17	11	28	19
MS2	3	2	5	2
MS3	11	8	19	4
MS4	6	6	12	5
MS6	10	4	14	7
MS7	5	0	4	1
MS8	4	1	4	1
MS9	3	1	3	2
MS10	14	4	18	1
MS11	12	5	17	15
Total	85	42	124	57

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Table 3 Loci analysed in MS families.

Locus	Probe	Location	Enzyme(s)	No of alleles	Allele frequency	Reference
<i>DIS2</i>	hMF1	1pter→p31	<i>Pst</i> I	>50	*	20
<i>DIS57</i>	pYNZ2	1pter→p31	<i>Pst</i> I	>10	*	21
<i>Rh</i>		1p36.2→p34		3	0.60 0.20 0.20	20
<i>PND</i>	pJA119	1p36	<i>Taq</i> I	3	0.93 0.05 0.02	22
	pJA110	1p36	<i>Bgl</i> II	2	0.89 0.11	
<i>DIS47</i>	CRI-LA336	1p35→p22	<i>Rsa</i> I	>20	*	23
<i>GLUT</i>	gt2-2	1p35→p31	<i>Xba</i> I	2	0.42 0.58	24
<i>DIS44</i>	CRI-R629	1p35→p31.3	<i>Hind</i> III	2	0.58 0.42	23
<i>DIS38</i>	CRI-S182	1p22→p13	<i>Msp</i> I	3	0.45 0.45 0.10	23
<i>DIS53</i>	CRI-L673	1q23→q25	<i>Msp</i> I	2	0.10 0.90	23
<i>D2S28</i>	CRI-R40	2p16→p14	<i>Bgl</i> III	3	0.05 0.55 0.45	23
<i>D2S21</i>	CRI-L22	2q11→q13	<i>Taq</i> I	3	0.09 0.33 0.58	23
<i>D2S44</i>	pYNH24	2q12→q32	<i>Pst</i> I	>20	*	21
<i>D2S39</i>	CRI-L586	2q22→q24	<i>Msp</i> I	2	0.77 0.23	23
<i>D2S6</i>	pXG-18	2q32→q36	<i>Taq</i> I	2	0.25 0.75	25
<i>D2S3</i>	p2-96	2q35→q37	<i>Taq</i> I	2	0.32 0.68	26
<i>COL6A3</i>	p24	2q37	<i>Nco</i> I	2	0.50	27
<i>D3S17</i>	CRI-L892	3p24→p23	<i>Taq</i> I	3	0.63 0.25 0.12	23
<i>D3S12</i>	CRI-R59	3p21.3→p21.1	<i>Msp</i> I	2	0.50	23
<i>D3S13</i>	CRI-R96	3q11→q12	<i>Msp</i> I	3	0.25 0.50 0.25	23
<i>D3S14</i>	CRI-R208	3q21→q22	<i>Bgl</i> III	2	0.64 0.36	23
			<i>Msp</i> I	3	0.11 0.39 0.50	23
<i>D4S101</i>	CRI-R227	4p24→p27	<i>Eco</i> RI	2	0.33 0.67	23
<i>D4S107</i>	CRI-L1408-A	4p16	<i>Msp</i> I	2	0.76 0.24	23
	CRI-L1408-B			2	0.33 0.67	
<i>D4S103</i>	CRI-L1190-A	4p13→p11	<i>Msp</i> I	2	0.43 0.57	23
	CRI-L1190-B			2	0.33 0.67	
<i>D5S51</i>	CRI-L1155	5q14→q21	<i>Hinc</i> II	2	0.90 0.10	23
<i>D6S33</i>	CRI-L994	6q16→q21	<i>Taq</i> I	2	0.15 0.85	23
<i>D7S62</i>	CRI-L1020	7pter→q22	<i>Hind</i> III	2	0.42 0.58	23
<i>D7S15</i>	CRI-L917	7q21→q22	<i>Hinc</i> II	2	0.48 0.52	23
<i>COL1A2</i>	NJ142	7q21.3→q22.1	<i>Eco</i> RI	2	0.90 0.10	13
<i>D7S54</i>	CRI-L281	7q35→qter	<i>Taq</i> I	4	0.25	23
<i>D10S12</i>	CRI-L368	10q24.1	<i>Hind</i> III	4	0.19 0.15 0.12 0.54	23
<i>D11S134</i>	CRI-L834	11p14	<i>Msp</i> I	2	0.55 0.45	23
<i>D11S141</i>	CRI-L762-A	11q13.5→q14.2	<i>Taq</i> I	2	0.88 0.12	23
	CRI-L762-B			2	0.43 0.57	

(Cont.)

Table 3 cont.

Locus	Probe	Location	Enzyme(s)	No of alleles	Allele frequency	Reference
<i>D11S137</i>	CRI-R83	11q14.3→q22.3	<i>MspI</i>	2	0.56 0.44	23
<i>D11S132</i>	CRI-L424	11q22→q23	<i>HindIII</i>	2	0.59 0.41	23
<i>COL2A1</i>	Pis2/8-0	12q14.3	<i>HindIII</i>	2	0.54 0.46	28
<i>D14S27</i>	CRI-L1013	14q31	<i>BglII</i>	2	0.50	23
<i>D15S49</i>	CRI-L1204	15q15→q23	<i>MspI</i>	2	0.39 0.61	23
<i>D17S37</i>	CRI-L581	17q21	<i>TaqI</i>	2	0.25 0.75	23
<i>COL1A1</i>	p2FC6	17q21.3→q22	<i>RsaI</i>	2	0.83 0.17	29
<i>D17S79</i>	pAC256	17q24→q25	<i>PstI</i>	>20	*	21

*VNTR probes.

subluxed. Blood samples were obtained from affected subjects and their unaffected relatives for DNA extraction and, in some, for lymphoblastoid transformation. Plasma, serum, and cell layer fractions were also obtained in one of the families. Before including a family in the testing panel, DNA from all subjects was evaluated to verify stated familial relationships using probes for at least two of the highly polymorphic loci that have been reported by Balazs *et al.*¹⁸ Table 2 summarises the structure of the 10 pedigrees initially collected for this study.

The power of a linkage study using these families was then determined using the programs SIMLINK and LODSTAT.¹⁹ Simulation using a two allele system with frequencies of 0.6 and 0.4 (heterozygosity=0.48) identified four families that would each yield a mean maximum lod score of less than 1.0 when the true recombination fraction was zero. In addition, these same families each had a mean estimated probability of greater than 0.5 of having a lod score greater than 0.0 at $\theta=0.1$ when the marker was actually unlinked. These four families were removed from further analyses and the power of the remaining six families was estimated. Under the same conditions as above, a lod score of 3.0 or greater should be obtained over 90% of the time when the true recombination fraction is 0.05 and 70% of the time when the true recombination fraction is 0.1. Moreover, with these six families an unlinked marker will generally be excluded for a recombination fraction of 0.18. For those marker loci having heterozygosities greater than 0.48, the distance at which linkage can be established or can be ruled out will be greater than the above estimates. These six families (MS1, MS3, MS4, MS6, MS10, MS11) were used for the majority of the linkage studies reported here.

MARKER LOCI TESTED

A total of 40 marker loci (39 RFLP loci and the Rh

blood group) were chosen for analysis of linkage with MS. The marker loci included candidate genes (COL1A1, COL1A2, COL2A1, COL6A3) and random probes located on chromosomes 1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 14, 15, and 17. Several loci on chromosome 1 were chosen for their proximity to the Rh locus, as Mace¹⁰ had reported a positive lod score for the Rh blood group in which the maximum lod score (1.25) was at $\theta=0.24$. The remaining random marker loci were chosen for their high heterozygosity (greater than 0.3) and simple patterns of alleles. The informative loci analysed in this study are described in table 3.²⁰⁻²⁹ Whenever possible, for those loci with more than one system or enzyme, haplotypes were used in the analysis.

RFLP ANALYSIS

High molecular weight DNA was extracted from peripheral blood or Epstein-Barr virus transformed lymphoblasts. The DNA was digested with the appropriate restriction enzymes, fractionated on 0.7 to 1.5% agarose gels, transferred to nylon membranes and then hybridised to DNA probes labelled with ³²P by either nick translation or the random primer technique, as described elsewhere.¹³ The results were analysed with PC adapted versions of LIPED³⁰ and MLINK.³¹ The Marfan locus was defined as having full penetrance.

Results

All probes used gave informative results in at least one family. The approximate chromosomal location is known for all of these probes. Eight of these families were also typed for the Rh blood group. None of the marker loci was linked to the MS locus at $\theta=0.0$. At $\theta=0.1$, 26 out of the 40 loci had a lod score of -2.0 or less. Linkage could be ruled out at $\theta=0.05$ for an additional four loci. Linkage of MS to various markers in the region surrounding the Rh locus was

Table 4 Lod scores at various recombination values of θ .

Locus	0.001	0.05	0.10	0.20	0.30	0.40
<i>D1S2</i>	-56.20	-17.02	-10.50	-4.72	-2.03	-0.67
<i>D1S57</i>	-38.35	-11.63	-7.25	-3.47	-1.64	-0.59
<i>Rh</i>	-32.63	-8.25	-4.26	-1.15	-0.10	0.14
<i>PND</i>	-15.96	-5.01	-2.85	-1.04	-0.29	-0.06
<i>D1S47</i>	-25.30	-7.68	-4.69	-1.98	-0.81	-0.26
<i>GLUT</i>	-8.10	-1.54	-0.60	0.03	0.16	0.11
<i>D1S44</i>	-10.10	-1.93	-0.78	0.03	0.16	0.11
<i>D1S38</i>	-20.55	-5.57	-3.20	-1.24	-0.46	-0.16
<i>D1S53</i>	-27.03	-7.73	-4.58	-1.87	-0.70	-0.18
<i>D2S28</i>	-8.01	-2.85	-1.93	-1.06	-0.60	-0.27
<i>D2S21</i>	-20.35	-7.03	-4.52	-2.37	-1.22	-0.49
<i>D2S44</i>	-69.87	-21.37	-13.23	-5.99	-2.61	-0.86
<i>D2S39</i>	-24.36	-7.66	-4.95	-2.53	-1.30	-0.50
<i>D2S6</i>	-12.25	-3.78	-2.33	-1.01	-0.37	-0.09
<i>D2S3</i>	-14.63	-4.68	-2.96	-1.40	-0.63	-0.21
<i>COL6A3</i>	-4.58	-1.42	-0.85	-0.36	-0.13	-0.03
<i>D3S17</i>	-1.98	-0.35	-0.12	0.01	0.02	0.01
<i>D3S12</i>	-40.40	-12.19	-7.29	-2.95	-1.02	-0.19
<i>D3S13</i>	-5.44	-0.60	-0.05	0.43	0.38	0.16
<i>D3S14</i>	-28.16	-6.64	-3.38	-0.89	-0.14	-0.04
<i>D4S101</i>	-7.52	-1.98	-1.46	-0.63	-0.23	-0.05
<i>D4S107</i>	-25.11	-6.95	-4.02	-1.59	-0.54	-0.11
<i>D4S103</i>	-15.63	-4.07	-2.30	-0.79	-0.15	0.08
<i>D5S51</i>	-8.18	-2.74	-1.72	-0.76	-0.31	-0.08
<i>D6S3</i>	-5.36	-1.92	-1.29	-0.67	-0.33	-0.12
<i>D7S62</i>	-12.49	-2.64	-1.12	-0.05	0.17	0.10
<i>D7S15</i>	-12.22	-3.78	-2.35	-1.06	-0.44	-0.12
<i>COL1A2</i>	-10.98	-2.85	-1.54	-0.49	-0.11	-0.01
<i>D7S54</i>	-55.77	-17.21	-10.86	-5.15	-2.39	-0.83
<i>D10S12</i>	-50.28	-16.27	-10.35	-5.08	-2.52	-0.98
<i>D11S134</i>	-26.18	-7.84	-4.88	-2.27	-1.04	-0.40
<i>D11S141</i>	-25.69	-7.58	-4.60	-1.98	-0.78	-0.20
<i>D11S137</i>	-14.37	-4.38	-2.78	-1.38	-0.71	-0.30
<i>D11S132</i>	-16.17	-4.56	-2.75	-1.24	-0.60	-0.27
<i>COL2A1</i>	-32.07	-10.49	-6.81	-3.38	-1.62	-0.59
<i>D14S27</i>	-0.30	-0.23	-0.17	-0.09	-0.04	-0.01
<i>D15S49</i>	-9.61	-1.25	-0.04	0.67	0.65	0.35
<i>D17S37</i>	-2.17	-0.29	-0.03	0.21	0.18	0.09
<i>COL1A1</i>	-17.14	-5.57	-3.51	-1.73	-0.86	-0.35
<i>D17S79</i>	-42.23	-10.03	-4.72	-0.56	0.74	0.77

excluded, thus suggesting that the results obtained by Mace¹⁰ were probably spurious. Lod scores with the marker loci are given in table 4.

Discussion

In this study, we ascertained classical Marfan syndrome segregating in 10 three generation families. Using RFLPs associated with candidate genes and random probes on 13 autosomes, we have confirmed exclusion of linkage of *COL1A1* and *COL2A1* at $\theta=0.15$ and 0.25 respectively, and have observed recombination of the MS locus with *COL1A2* and *COL6A3*. We have presented data that can form the basis for an exclusion map of the MS locus, as summarised by Blanton *et al* in this issue of the Journal. In addition, despite the reported wide variability in expression of the MS gene, our study, to date, does not indicate any genetic heterogeneity in our 10 families.

So far, the major fibrillar collagen genes have been excluded as the basic defect in MS. Additional connective tissue matrix components need to be studied as candidate loci. In conjunction with the

candidate locus approach, we hope that using random probes will facilitate establishing chromosomal localisation of the MS locus and lead the way to mapping of the locus.

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