Linkage analysis of five fibrillar collagen loci in a large French Marfan syndrome family

C Boileau, G Jondeau, C Bonaiti, M Coulon, G Delorme, O Dubourg, J-P Bourdarias, C Junien

Abstract

Marfan syndrome consists of a group of dominantly inherited disorders of connective tissue with wide clinical variability. Using the candidate gene approach, we have attempted to map the gene defect in a large French Marfan syndrome family with no ocular manifestations. We performed linkage studies with polymorphic probes for five structural procollagen genes. The data obtained exclude linkage of Marfan syndrome to the two major fibrillar collagen (COL1A1, COL1A2, and COL2A1) genes. These results confirm previously published data obtained from smaller pedigrees. A small positive lod score ($Z=0.99$, $\theta=0.00$) was obtained for the COL3A1-COL5A2 gene cluster located on chromosome 2.

Marfan syndrome (MS), an autosomal dominant disease, is associated with ocular, cardiovascular, and skeletal abnormalities. Variability of clinical expression is a hallmark of the disorder. The incidence of MS has been estimated at 1/25 000 and about one quarter of the cases are thought to result from new mutations.¹

Although MS was the 'founding member' of inherited matrix disorders,² it has remained an unmapped disease. Numerous biochemical reports have implied that it could be an inborn error of protein metabolism, particularly in collagen or elastin.

However, the underlying pathogenesis of MS remains unknown. One approach to identifying the mutant locus is by the 'candidate gene approach': genetic linkage studies are performed in multiplex families with candidate loci encoding components of the extracellular matrix. Several groups have already published exclusion data obtained with the major candidates, the fibrillar procollagen genes (for review see Francomano *et al*³). However, clinical variability between MS families is the rule and could result from defects in different connective tissue components. Therefore, it is important to confirm, in each pedigree, previously reported exclusions, for there always remains the possibility that the mutant gene may be one of the genes already excluded. Using an exceptionally large French kindred with MS, we have attempted to map the gene defect using the same approach.

Material and methods

PATIENTS

Forty-nine members of a large French pedigree (figure), comprising to date 173 subjects with at least four generations affected, were tested: three from generation II (2, 8, 10), 20 from generation III (2, 3, 4, 5, 10, 11, 12, 13, 16, 20, 29, 33, 35, 36, 37, 40, 41, 43, 44, 52), and 26 from generation IV (7, 8, 9, 10, 13, 14, 17, 18, 24, 26, 29, 30, 32, 35, 42, 44, 46, 49, 51, 52, 53, 54, 55, 57, 58, 59). The family was ascertained by one of us (J-PB) through the Department of Cardiology, Hôpital Ambroise Paré. This family had been under medical surveillance since 1977 after the death of a second sib from ruptured dissecting aneurysm of the ascending aorta. Histological studies in two patients showed that elastic fibres of the media were separated and disrupted by collagen fibrils and mucoid material. Of the 37 subjects (mean age 28.3 years) in two generations who underwent physical examination, echocardiography, and slit lamp examination, 19 were considered to be genetically affected since they had manifestations in two out of the three systems involved, namely skeletal and cardiovascular.⁴ At least one skeletal anomaly was observed in 89% of them: arachnodactyly (38.9%), narrow arched palate (33.3%), pectus excavatum (16.7%), and scoliosis (16.7%). Increased lower-upper

Laboratoire de Biochimie et de Génétique Moléculaire, Hôpital Ambroise Paré, 92000 Boulogne, France.
C Boileau, C Junien

INSERM U73, Château de Longchamp, Bois de Boulogne, 75016 Paris, France.
C Boileau, M Coulon, C Junien

Service de Cardiologie, Hôpital Ambroise Paré, 92000 Boulogne, France.
G Jondeau, G Delorme, O Dubourg, J-P Bourdarias

INSERM U155, Château de Longchamp, 75016 Paris, France.
C Bonaiti

Correspondence to Dr Junien, Paris.

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Family pedigree. Filled symbols indicate affected subjects; partially filled symbols indicate either an obligate carrier or persons with insufficient evidence for diagnosis. On linkage analysis only the obligate carrier III·13 was considered as affected.

body segment ratio, tall stature, and increased arm span were observed in all affected subjects. Two dimensional echocardiography and Doppler studies showed mitral valve prolapse (66·4%) associated with holosystolic mitral regurgitation (26·3%),⁵ tricuspid valve prolapse (26·3%), and dilatation of the ascending aorta (58%).⁶ None of the affected subjects examined had ectopia lentis.

RFLP ANALYSIS

DNA was prepared from peripheral blood leucocytes or Epstein-Barr virus transformed lymphoblastoid cell lines as previously described.⁷ Restriction enzyme digests were performed according to manufacturers' instructions and fragments were resolved by 0·8% agarose gel electrophoresis. DNA was transferred to Hybond-N membranes (Amersham) that were (pre)

Table 1 Probes and RFLPs.

Gene	Chromosomal localisation	Probe	Restriction enzyme	Allelic fragments (kb)	Allele frequency	References
COL1A1	17q21.3→q22	XHO	<i>RsaI</i>	3·6 2·6	0·14 0·86	9
COL1A2	7q21.3→q22.1	Hf32	<i>RsaI</i>	2·9 2·1+0·8	0·11 0·89	10
COL2A1	12q14.3	pgHColIIA	<i>HindIII</i>	14·0 7·0	0·65 0·35	11
COL3A1	2q14→q32	pPB1	<i>EcoRI</i>	2·1 1·7	0·39 0·61	R Dagleish, personal communication
		pPB1	<i>AvaII</i>	6·2 4·5	0·74 0·26	12
COL5A2	2q14→q32	NH2	<i>MspI</i>	13·0 9·0	0·15 0·85	13

Table 2 Detailed results of pairwise linkage analysis of MS and collagen gene markers.

Locus 2			Lod score at recombination fractions of:									
Locus 1	Gene	Probe/enzyme	0·000	0·001	0·01	0·05	0·10	0·15	0·20	0·25	0·30	0·40
MS	COL1A1	XHO/ <i>RsaI</i>	-4·666	-2·115	-1·130	-0·489	-0·258	-0·148	-0·087	-0·052	-0·032	-0·014
MS	COL1A2	Hf32/ <i>RsaI</i>	-5·626	-4·102	-2·242	-0·751	-0·173	0·080	0·192	0·226	0·215	0·123
MS	COL2A1	pgHColIIA/ <i>HindIII</i>	-4·971	-4·972	-4·692	-2·693	-1·659	-1·075	-0·693	-0·431	-0·250	-0·052
MS	COL3A1	pPB1/ <i>EcoRI</i>	-0·012	-0·012	-0·012	-0·009	-0·007	-0·005	-0·004	-0·003	-0·002	-0·000
MS	COL3A1	pPB1/ <i>AvaII</i>	-0·005	-0·005	-0·005	-0·003	-0·002	-0·001	-0·001	-0·000	-0·000	-0·000
MS	COL5A2	NH2/ <i>MspI</i>	0·992	0·991	0·981	0·925	0·836	0·731	0·614	0·489	0·360	0·128

hybridised, washed, and exposed as previously reported.⁷ Probes were labelled with ³²P dCTP by random priming⁸ to high specific activity. Detailed information on the molecular probes and RFLPs tested is given in table 1.⁹⁻¹³

LINKAGE ANALYSIS

Linkage analysis was performed with the program LINKAGE.¹⁴ A frequency for the disease allele of 0·00002 and a penetrance of 90% were used.

Results

Forty-nine members of the pedigree, including 18 affected subjects, were first screened with probes from four procollagen candidate genes: COL1A1, COL1A2, COL2A1, and COL3A1. Detailed results of pairwise linkage analyses between MS and each of the RFLPs are shown in table 2.

The family was informative for the COL1A1, COL1A2, and COL2A1 markers. Strong evidence against linkage was found for these genes with respective maximum lod scores of -4·66, -5·62, and -4·97 at $\theta=0·00$. For COL3A1, the two RFLPs tested were not informative. However, since the COL3A1 and the COL5A2 genes both map to 2q14→q32^{15,16} and are tightly linked,¹⁷ probe NH2,

which detects an *MspI* RFLP in COL5A2, was used. It generated a small positive lod score ($Z=0·99$, $\theta=0·00$).

Discussion

Although a defect in one of the major collagen genes has long been thought to be implicated in the pathogenesis of MS, our results exclude linkage between the disease and either the type I or type II procollagen genes. Other authors have also published exclusion data between the disease and these loci.^{3,18-20} In these studies, one or a few much smaller and clinically heterogeneous pedigrees were reported. Because of the great variability observed between MS families, which could be the result of heterogeneous underlying defects, our results should be compared only with data originating from phenotypically similar cases. (1) Francomano *et al*³ reported five out of 12 pedigrees without ectopia lentis. Among them, exclusion ($Z=-\infty$, $\theta=0·00$) of the three loci tested (*COL1A2*, *COL2A1*, and *COL3A1*) was obtained in only one family. (2) Of the families studied by Ogilvie *et al*,²⁰ only two did not have ocular involvement. Neither of the type I procollagen genes could be clearly excluded in the two families. No data concerning linkage between MS and *COL2A1* or *COL3A2* were reported. Our results, obtained in a

single large pedigree, confirm the exclusion data for collagens type I and II.

Linkage between MS and the COL3A1–COL5A2 gene cluster was not fully investigated in either of the previous studies. The small positive result obtained in our study cannot be investigated further by the candidate gene approach since no other RFLPs than the ones reported here have been described for the cluster. Interestingly, the long arm of chromosome 2, along with the COL3A1 and COL5A2 genes, harbours other candidate genes such as elastin (2q31→qter), COL6A3 (2q37), and fibronectin (2q34→q36). Further linkage studies between MS and the three candidate loci, as well as anonymous polymorphic DNA markers closely linked to the cluster in 2q14→q32, should elucidate the ambiguous results presented here.

The collagen candidate gene approach has now been used by many groups to map the gene defect in connective tissue disorders. It has only been successful in several types of osteogenesis imperfecta^{9, 21} and Ehlers-Danlos syndrome.²² In the case of MS no conclusive data have been reported using this approach. Though there are other candidate genes among the components of the extracellular matrix, it is likely that the best approach to the Marfan locus will now be achieved by linkage analysis with evenly dispersed anonymous DNA markers.

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