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, θ=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 1/20 000 and about one quarter of the cases are thought to result from new mutations. Although MS was the 'founder member' of inherited matrix disorders, 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). 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 aeurysm 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. 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
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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%), tricuspid valve prolapse (26-3%), and dilatation of the ascending aorta (58%). 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. 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)
hybridised, washed, and exposed as previously reported. Probes were labelled with \(^{32}\)P dCTP by random priming to high specific activity. Detailed information on the molecular probes and RFLPs tested is given in table 1.\(^6\)-\(^{13}\)

**Table 1** Probes and RFLPs.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal localisation</th>
<th>Probe</th>
<th>Restriction enzyme</th>
<th>Allellic fragments (kb)</th>
<th>Allele frequency</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL1A1</td>
<td>17q21.3-q22</td>
<td>XHO</td>
<td>RsaI</td>
<td>3.6</td>
<td>0.14</td>
<td>9</td>
</tr>
<tr>
<td>COL1A2</td>
<td>7q21.3-q22.1</td>
<td>H32</td>
<td>RsaI</td>
<td>2.6</td>
<td>0.86</td>
<td>10</td>
</tr>
<tr>
<td>COL2A1</td>
<td>12q14.3</td>
<td>pGHeIIA</td>
<td>HindIII</td>
<td>2.1</td>
<td>0.65</td>
<td>11</td>
</tr>
<tr>
<td>COL3A1</td>
<td>2q14-q32</td>
<td>pPB1</td>
<td>EcoRI</td>
<td>1.7</td>
<td>0.61</td>
<td>R Dalgleish, personal communication</td>
</tr>
<tr>
<td>COL5A2</td>
<td>2q14-q32</td>
<td>NH2</td>
<td>MspI</td>
<td>9.0</td>
<td>0.85</td>
<td>13</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Locus 2</th>
<th>Gene</th>
<th>Probe/enzyme</th>
<th>Lod score at recombination fractions of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0·000</td>
<td>0·001</td>
</tr>
<tr>
<td></td>
<td>COL1A1</td>
<td>XHO/RsaI</td>
<td>-4·66</td>
</tr>
<tr>
<td></td>
<td>COL1A2</td>
<td>H32/RsaI</td>
<td>-5·626</td>
</tr>
<tr>
<td></td>
<td>COL2A1</td>
<td>pGHeIIA/HindIII</td>
<td>-4·971</td>
</tr>
<tr>
<td></td>
<td>COL3A1</td>
<td>pPB1/EcoRI</td>
<td>-0·012</td>
</tr>
<tr>
<td></td>
<td>COL3A1</td>
<td>pPB1/AvaII</td>
<td>-0·005</td>
</tr>
<tr>
<td></td>
<td>COL5A2</td>
<td>NH2/MspI</td>
<td>0·992</td>
</tr>
</tbody>
</table>

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 θ=0·00. For COL3A1, the two RFLPs tested were not informative. However, since the COL3A1 and the COL5A2 genes both map to 2q14→q32\(^{15}\) 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, θ=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.\(^3\)\(^\text{18-20}\) 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 phenotype-similar cases. (1) Francomano et al\(^5\) reported five out of 12 pedigrees without ectopia lentis. Among them, exclusion (Z=−∞, θ=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 collagen types 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 and Ehlers-Danlos syndrome. 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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15. Emanuel BS, Cannizzaro LA, Seyers JM, Meyers JC. Human α1(III) and α2(V) procollagen genes are located on the long arm of chromosome 2. Proc Natl Acad Sci USA 1985;82:3385–9.
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