Exclusion of the α2(I) and α1(III) collagen genes as the mutant loci in a Marfan syndrome family

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SUMMARY The inheritance of restriction fragment length polymorphisms for two fibrillar collagen genes (COL1A2 and COL3A1) has been studied in a large Marfan syndrome kindred. We are able to show discordant segregation between the Marfan syndrome and each of the two collagen gene markers.

In recent years evidence has accumulated to show that the basic molecular defect in many inherited disorders of connective tissue is due to a mutation in one of the collagen genes. The Marfan syndrome is a connective tissue disorder about which, as yet, there is little biochemical information. It is transmitted as an autosomal dominant trait with wide variability of expression. About 5% of cases are new mutations and quoted prevalence rates range widely from 1:5/100 000 to 4/100 000. There is, in addition, a marked phenotypic heterogeneity and these rates may greatly underestimate the true prevalence.

In its typical form the Marfan syndrome consists of skeletal disproportion, arachnodactyly, ocular disorders (myopia and ectopic lens), and cardiovascular problems (principally aortic incompetence and dissecting aneurysm of the ascending aorta). Early death frequently results from these cardiological abnormalities, both in the patients with the fully penetrant syndrome and in those who have no other obvious clinical signs of the disease (formes frustes). It has been shown that there is no correlation between the severity of the cardiac and the oculoskeletal abnormalities.

With increased understanding of the progressive nature of the condition, the prognosis for affected patients could be improved by the use of beta blocker medication, annual screening of the aortic root diameter, and elective prophylactic surgery. Although it is desirable in any particular subject with the Marfan syndrome to define the precise biochemical abnormality of their condition, it is of greater urgency to identify those who are at risk.

The availability of gene clones for connective tissue components has enabled us to study the segregation of the disease in families using restriction fragment length polymorphisms (RFLPs) in candidate genes. Types I and III collagen are major structural proteins in man. Type I collagen has already been implicated as the defective component in this disorder in a patient producing a longer pro-α2 chain of type I collagen. The α2(I) procollagen α1 chain is encoded at a single locus (COLIA2) on chromosome 7.

Type III collagen is a prominent component of both the media and adventitia of the wall of the aorta and is formed by three identical α1 chains with subunits encoded at a single locus (COL3A1) on chromosome 2.

Here we describe the results using DNA sequence polymorphisms in one of our Marfan families, to test the possibility that the causal mutation is within, or close to, either of these candidate genes.

Patients and methods

The Marfan syndrome was first diagnosed in this family (fig 1) in 1965 when it was noted that the grandmother (I.3) had bilateral dislocation of the lens. She was not, however, typically Marfanoid in appearance and the diagnosis was not made until she was 64 years of age.

On examination, it was found that four of her children had ectopic lenses and each of them also presented with skeletal abnormalities. In 1983, II.6 died suddenly of a ruptured dissecting aneurysm of the ascending arch of the aorta. This son was 215 cm in height and showed all the manifestations of the syndrome. The younger affected members (III.4, 4.6)
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III.6, III.18, III.19, and III.20) all have ectopic lenses and varying degrees of skeletal disproportion. III.4 has early cardiovascular signs.

DNA was prepared by standard procedures from blood collected into EDTA. Samples of approximately 4 mg were digested with restriction enzymes, fractionated in 0.8% agarose, and blotted onto nylon membranes (Hybond-N, Amersham, UK).

Hybridisation probes were prepared using cloned DNA fragments isolated free of vector DNA. The fragments were radiolabelled to a specific activity of approximately $1.5 \times 10^9$ dpm $\mu$g$^{-1}$ using the oligo labelling method. Hybridisations were carried out in $3 \times$ SSC, $2 \times$ Denhardt's solution, 200 $\mu$g ml$^{-1}$ sonicated and denatured herring sperm DNA, 6% polyethylene glycol 8000, 0.1% SDS at 65°C for 16 to 18 hours at a probe concentration of 0.5 ng ml$^{-1}$. Filters were washed to a final stringency of $0.5 \times$ SSC, 0.1% SDS at 65°C and autoradiographed overnight on Kodak X-Omat AR film with intensifying screens at $-80°C$.

Results

Two collagen gene RFLPs were used to study the Marfan syndrome in the pedigree shown in fig 1.

$\alpha_2$(I) gene RFLP

An Rsal RFLP in the pro $\alpha_2$(I) collagen gene has previously been described in detail. The hybridisation probe used was the 1.2 kb EcoRI fragment from the genomic clone $\lambda$Hproa2(I)$^{-1}$. The polymorphic fragments detected are 2.9 kb and 2.1 kb as indicated in fig 2. The more common 2.1 kb allele is termed A and the 2.9 kb allele termed B. Results are shown in figs 1 and 2.

Subject II.16 had alleles A and B and passed allele A to subjects III.19 and III.20, while allele B was passed on to subject III.18. Subject II.17 passed allele A to all of her offspring. Since II.16, III.18, III.19, and III.20 all have Marfan syndrome, the $\alpha_2$(I) is thus excluded as the mutant locus in this pedigree. If it were the mutant locus, then II.16 would have passed on the same allele (either A or B) to all of his offspring.

$\alpha_1$(III) gene RFLP

An AvaII RFLP close to the pro $\alpha_1$(III) collagen gene has previously been reported. A subcloned region
(pIII-33-600) of a type III collagen cDNA reveals polymorphic DNA fragments of 6.2 kb and 4.5 kb which are termed alleles C and D respectively (fig 3).

Affected subject II.3 passed on allele C to III.2 and III.6 and passed on allele D to III.3 and III.4. Subjects III.2 and III.3 are normal whereas III.4 and III.6 have Marfan syndrome. III.4 and III.6 inherited different pro α1(III) alleles from their affected mother, so therefore the pro α1(III) gene is not the mutant locus in this Marfan pedigree.

Discussion

The present study indicates that the mutation causing Marfan syndrome in this family is not in, or closely linked to, the pro α2(I) or pro α1(III) collagen genes, both of which had been likely candidate loci. The discordances are based entirely on comparison of the genotypes of affected family members so that questions of incomplete penetrance do not arise. The probability of recombination between the marker RFLPs and the loci being tested as candidates for the mutation is extremely low due to their proximity. The results are in agreement with a preliminary report describing exclusion of the pro α2(I) collagen gene, but are clearly at odds with the finding of defective pro α2(I) collagen chains in another case of Marfan syndrome. In that particular case an insertion of 38 base pairs was detected in an intron of the pro α2(I) gene. We have shown that the insert is a common variant found in normal subjects.

The possibility of non-paternity in the informative matings (II.3 × II.4 and II.16 × II.17) could give rise to unfair exclusions of genes. In the latter case such non-paternity is unlikely since the father is the affected parent. In the former, however, the possibility of non-paternity is higher but we have confirmed correct paternity by minisatellite fingerprinting of DNA from the relevant subjects (data not shown).

The results presented here derive from a single unlinked pedigree and the possibility remains of several unlinked Marfan syndrome loci. To resolve this, a number of other Marfan pedigrees are being studied at present.

Having excluded two loci, it is now necessary to consider other candidates. Although a number of RFLP markers exist for the type II collagen gene, it is unlikely that it is the disease locus since type II collagen is expressed primarily in cartilage and vitreous humour. The recent discovery of RFLPs within, and adjacent to, the pro α1(I) collagen gene will also allow the study of that locus as a candidate. The mutant locus may in fact be the gene for some connective tissue component other than collagen or for an enzyme involved in connective tissue modelling.

An alternative approach that does not rely on the availability of such cloned genes entails the use of cloned probes for hypervariable minisatellite sequences which produce individual specific DNA 'fingerprints'. Such analyses can be used to study the segregation of multiple informative loci in large human pedigrees, to search for linkage between hypervariable DNA fragments and disease loci, and to provide a means of analysing the Marfan syndrome mutation without necessarily resorting to cloning genes for further connective tissue components.

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References

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7 Committee on mutagenicity of chemicals in foods, consumer products and of the environment. Guidelines for the testing of chemicals for mutagenicity. London: HMSO, 1981.


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