Exclusion of the $\alpha 2(I)$ and $\alpha 1(III)$ collagen genes as the mutant loci in a Marfan syndrome family

RAYMOND DALGLEISH*, J ROSS HAWKINS*, AND MARION KESTON†

From *the Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH; and †the MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU.

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.^{1,2} The Marfan syndrome³⁻⁵ 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 000⁶ to 4/100 000.⁷ 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).⁸ It has been shown that there is no correlation between the severity of the cardiac and the oculoskeletal abnormalities.⁹

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.¹⁰ 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 $\alpha 2$ chain of type I collagen.¹¹ The $\alpha 2(I)$ procollagen chain is encoded at a single locus (*COL1A2*) on chromosome 7.¹²

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 α chain subunits encoded at a single locus (*COL3A1*) on chromosome 2.¹³

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.8 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

Received for publication 23 May 1986.

Revised version accepted for publication 15 August 1986.

FIG 1 Family pedigree. The letters A, B, C, and D below symbols indicate collagen gene alleles detected (see Results and figs 2 and 3). Solid symbols indicate affected subjects.

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 μ g 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 1.5×10^9 dpm μ g $^{-1}$ using the oligo labelling method.¹⁴ Hybridisations were carried out in $3 \times$ SSC, $2 \times$ Denhardt's solution, 200 μ 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 *RsaI* RFLP in the pro $\alpha 2(I)$ collagen gene has previously been described in detail.¹⁵ The hybridisation probe used was the 1.2 kb *EcoRI* fragment from the genomic clone λ Hpro $\alpha 2(I)$ -1.¹⁶ 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 has previously been reported.¹⁷ A subcloned region

FIG 2 Detection of the pro $\alpha 2(I)$ collagen gene *RsaI* RFLP. The bands at 2.1 and 2.9 kb are termed A and B, respectively.

FIG 3 Detection of the pro $\alpha 1$ (III) collagen gene *Avall* RFLP. The bands at 6.2 and 4.5 kb are termed C and D, respectively.

(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 $\alpha 1$ (III) alleles from their affected mother, so therefore the pro $\alpha 1$ (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 $\alpha 2$ (I) or pro $\alpha 1$ (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 $\alpha 2$ (I) collagen gene,¹⁸ but are clearly at odds with the finding of defective pro $\alpha 2$ (I) collagen chains in another case of Marfan syndrome.¹¹ In that particular case an insertion of 38 base pairs was detected in an intron of the pro $\alpha 2$ (I) gene.¹⁹ We have shown that the insert is a common variant found in normal subjects.²⁰

The possibility of non-paternity in the informative

matings (II.3 $\times$ II.4 and II.16 $\times$ 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 DNA²¹ from the relevant subjects (data not shown).

The results presented here derive from a single 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,²² 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 $\alpha 1$ (I) collagen gene²³ 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'.²¹ 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,²⁴ and to provide a means of analysing the Marfan syndrome mutation without necessarily resorting to cloning genes for further connective tissue components.

We would like to thank Dr W H Price, Head of Clinical Studies Section at the MRC Edinburgh and Dr J F Cullen, Consultant Ophthalmologist of the Edinburgh Royal Infirmary for their help; Ila Patel and Alec Jeffreys for help with the 'fingerprints'; and Sheila Mackley for typing the manuscript. JRH is supported by an SERC studentship.

References

- 1 Prockop DJ. Mutations in collagen genes. Consequences for rare and common diseases. *J Clin Invest* 1985;75:783-7.
- 2 Cheah KSE. Collagen genes and inherited connective tissue disease. *Biochem J* 1985;229:287-303.
- 3 Pyeritz RE, McKusick VA. The Marfan syndrome: diagnosis and management. *N Engl J Med* 1979;300:772-7.
- 4 Pyeritz RE, McKusick VA. Basic defects in the Marfan syndrome. *N Engl J Med* 1981;305:1011-2.
- 5 Pinnell SR, Murad S. Disorders of collagen. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, Goldstein JL, Brown MS,

- eds. *The metabolic basis of inherited disease*. 5th ed. New York: McGraw-Hill 1983:1425-49.
- ⁶ Lynas MA. Marfan's syndrome in northern Ireland: an account of thirteen families. *Ann Hum Genet* 1958;**22**:289-309.
- 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.
- ⁸ Murdoch JH, Walker BA, Halpern BL, Juzma JW, McKusick VA. Life expectancy and causes of death in the Marfan syndrome. *N Engl J Med* 1972;**286**:804-8.
- ⁹ Bruno L, Tredici S, Mangiavacchi M, Colombo V, Mazzotta GF, Sirtori CR. Cardiac, skeletal, and ocular abnormalities in patients with Marfan's syndrome and in their relatives. Comparison with the cardiac abnormalities in patients with kyphoscoliosis. *Br Heart J* 1984;**51**:220-30.
- ¹⁰ Gott VL, Pyeritz RE, Magovern GJ, Cameron DE, McKusick VA. Surgical treatment of aneurysms of the ascending aorta in the Marfan syndrome. *N Engl J Med* 1986;**314**:1070-4.
- ¹¹ Byers PH, Siegel RC, Peterson KE, et al. Marfan syndrome: abnormal $\alpha 2$ chain in type I collagen. *Proc Natl Acad Sci USA* 1981;**78**:7745-9.
- ¹² Solomon E, Hiorns L, Dalglish R, Tolstoshev P, Crystal R, Sykes B. Regional localization of the human $\alpha 2(I)$ collagen gene on chromosome 7 by molecular hybridization. *Cytogenet Cell Genet* 1983;**35**:64-6.
- ¹³ Solomon E, Hiorns LR, Spurr N, et al. Chromosomal assignments of the genes coding for human types II, III, and IV collagen: a dispersed gene family. *Proc Natl Acad Sci USA* 1985;**82**:3330-4.
- ¹⁴ Feinberg AP, Vogelstein B. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 1984;**137**:266-7.
- ¹⁵ Grobler-Rabic AF, Wallis G, Brebner DK, Beighton P, Bester AJ, Mathew CG. Detection of a high frequency RsaI polymorphism in the human pro $\alpha 2(I)$ collagen gene which is linked to an autosomal dominant form of osteogenesis imperfecta. *EMBO J* 1985;**4**:1745-8.
- ¹⁶ Tajima S, Ting JPY, Pinnell SR, Kaufman RE. Isolation and characterization of a human pro $\alpha 2(I)$ collagen gene segment. *J Invest Dermatol* 1984;**82**:265-9.
- ¹⁷ Dalglish R, Woodhouse M, Reeders S. An RFLP associated with the human type III collagen gene (COL3A1). *Nucleic Acids Res* 1985;**13**:4609.
- ¹⁸ Borresen AL, Bamforth S, Tsipouras P, Berg K. Studies of RFLPs at the pro $\alpha 2(I)$ collagen locus excludes close linkage to Marfan syndrome. Human Gene Mapping 8. *Cytogenet Cell Genet* 1985;**40**:585A.
- ¹⁹ Henke E, Leader M, Tajima S, Pinnell S, Kaufman R. A 38 base pair insertion in the pro $\alpha 2(I)$ collagen gene of a patient with Marfan syndrome. *J Cell Biochem* 1985;**27**:161-74.
- ²⁰ Dalglish R, Williams G, Hawkins JR. Length polymorphism in the pro $\alpha 2(I)$ collagen gene: an alternative explanation in a case of Marfan syndrome. *Hum Genet* 1986;**73**:91-2.
- ²¹ Jeffreys AJ, Wilson V, Thein SL. Individual-specific 'fingerprints' of human DNA. *Nature* 1985;**314**:67-73.
- ²² Sykes B, Smith R, Vipond S, Paterson C, Cheah K, Solomon E. Exclusion of the $\alpha 1(II)$ cartilage collagen gene as the mutant locus in type IA osteogenesis imperfecta. *J Med Genet* 1985;**22**:187-91.
- ²³ Sykes B, Ogilvie D, Wordsworth P, Anderson J, Jones N. Osteogenesis imperfecta is linked to both type I collagen structural genes. *Lancet* 1986;**ii**:69-72.
- ²⁴ Jeffreys AJ, Wilson V, Thein SL, Weatherall DJ, Ponder BAJ. DNA 'fingerprints' and segregation analysis of multiple markers in human pedigrees. *Am J Hum Genet* 1986;**39**:11-24.

Correspondence and requests for reprints to Dr R Dalglish, Department of Genetics, University of Leicester, Adrian Building, University Road, Leicester LE1 7RH.