

Exclusion of the α_1 (II) collagen structural gene as the mutant locus in type II Ehlers-Danlos syndrome

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SUMMARY We have used a high frequency site polymorphism within the human pro- α_1 (II) collagen gene (COL2A1) in order to examine the segregation of this gene within a large pedigree with type II Ehlers-Danlos syndrome (EDS). The EDS gene and the collagen gene segregate independently within the pedigree and therefore COL2A1 can be excluded as the mutant locus.

The Ehlers-Danlos syndrome (EDS) comprises a heterogeneous group of heritable disorders characterised by physical abnormalities in many systems, suggesting mechanically defective collagen.¹ Deficiencies of collagen processing enzymes have been described in some rare variants of the syndrome, but it seems likely that in others the causal mutations may lie within or close to the structural genes coding for collagen. In type II EDS the most striking features are excessively mobile joints, and hyperextensible, fragile skin which tends to bruise and to heal with wide paper thin scars.

A 300 base-pair (bp) deletion in an ' α_1 (I)-like' collagen gene has been reported in a West Indian father and son with EDS II, and on this basis it has been suggested that this deletion is the mutation responsible for the disorder.^{2,3} Sequence data have since shown that this gene encodes the pro- α_1 (II) chain of type II collagen which is the major collagen species in both hyaline and articular cartilage.⁹ Since type II collagen is not normally found in the skin and ligaments, the most obviously affected tissues in EDS II, this gene is an unlikely candidate for the mutant locus. Nevertheless, in the light of the proposals that a deletion in this gene causes the disease and because it is unwise to dismiss any gene on biochemical grounds alone, we examined its segregation in a large pedigree with EDS II. If this gene contains the mutation which causes the disease in this family, then it must cosegregate with the EDS

gene throughout the pedigree. Any examples of independent segregation exclude the type II collagen gene as the EDS locus.

Patients and methods

Fifty-three persons from a family with dominantly inherited type II EDS were examined for evidence of joint hypermobility, skin hyperextensibility, and abnormal scars, in order to define affected and unaffected members. Deoxyribonucleic acid (DNA) was prepared from frozen whole blood.⁴ 10 μ g aliquots were digested with the restriction endonucleases EcoRI and Hind III. EcoRI conveniently displays the 300 bp deletion when present, while Hind III reveals a high frequency polymorphism. The fragments were separated in agarose and transferred to nitrocellulose filters under standard conditions.⁵ The blots were then hybridised overnight at 42°C with ³²P-labelled whole cosmid cosH col 1, which contains the entire pro- α_1 (II) collagen gene.⁶ The hybridisation mix contained 3 \times SSC, 50% formamide, 5% dextran sulphate, 1 \times Denhardt's solution, 20 mg/l denatured salmon sperm DNA, and 20 mg/l denatured human placenta DNA to compete with repetitive sequences in the probe. Filters were washed to 0.1 \times SSC and 0.1% sodium dodecyl sulphate (SDS) at 65°C, air dried, and exposed to Fuji RX film.

The segregation of the EDS gene was evaluated by clinical examination of affected and unaffected individuals, whereas that of the α_1 (II) collagen gene was determined with a high frequency Hind III restriction site polymorphism as a genetic marker.⁷

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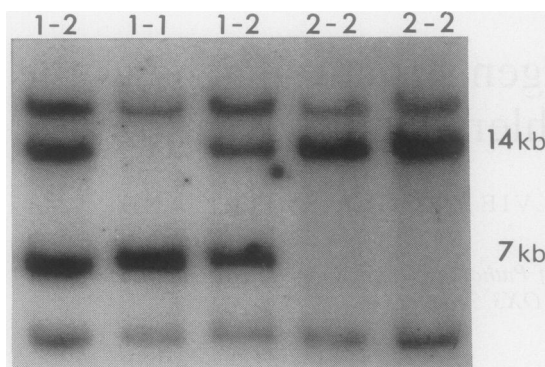


Fig. 1 *COL2A1* restriction fragment length polymorphism shown by digestion with *Hind* III. When the variable site is present the 14 kb fragment (allele 2) is cleaved into two fragments of 7 kb (allele 1). Individual genotypes are given above each track.

Results

Fig. 1 shows the pattern of fragments generated by digestion of DNA from members of this kindred with the restriction enzyme *Hind* III. The 14 kb fragment (allele 2) is cleaved into two 7 kb fragments (allele 1) when the restriction site is present. There are three possible patterns corresponding to the three diploid genotypes (1-1, 1-2, 2-2). When the genotypes of the pedigree members are used to analyse the segregation of the $\alpha_1(\text{II})$ gene three examples of discordant segregation of this gene and the EDS II gene emerge (Fig. 2).

(1) The genotype of IV.4 is 1-2, and she carries the EDS gene. She inherited the EDS gene and allele 2 from her homozygous (2-2) affected

father III.4. Thus if the $\alpha_1(\text{II})$ gene is the mutant locus, the EDS gene must be linked to allele 2. However, her son (V.2) has inherited the EDS gene and allele 1 rather than allele 2 from his mother.

- (2) The genotype of III.10 is 1-2. He has transmitted allele 1 to his sons IV.14 and IV.15 but the EDS gene to only one of them (IV.14).
- (3) III.10 has transmitted allele 2 to his daughter IV.12 and allele 1 to his son IV.15, but neither has inherited the EDS gene.

Since examples (2) and (3) involve clinically unaffected individuals, incomplete penetrance of the EDS gene could influence the results. However, there was no evidence of even mild disease in any of these individuals, so it is unlikely that they inherited the EDS gene. Example (1) is not influenced by this proviso, since the analysis depends only on obviously affected individuals.

Fig. 3 shows the *Eco*RI fragment pattern from members of the pedigree. There are no examples of a 300 bp deletion in the 4.3 kb fragment as reported in the family described by Pope *et al.*,³ which would be seen as a clear double band at this position. However, there is a barely discernible doublet in this fragment in individuals IV.20, V.7, and IV.24. Reference to the pedigree in Fig. 2 shows that one is affected (IV.20) and two are not (IV.24, V.7).

Discussion

A 300 bp deletion in the gene encoding $\alpha_1(\text{II})$ collagen has been proposed as the causal mutation in EDS II. We have been unable to confirm this in a large pedigree. There is a highly variable region just beyond the 3' end of this gene, and deletions of about 300 bp in this area are common in normal

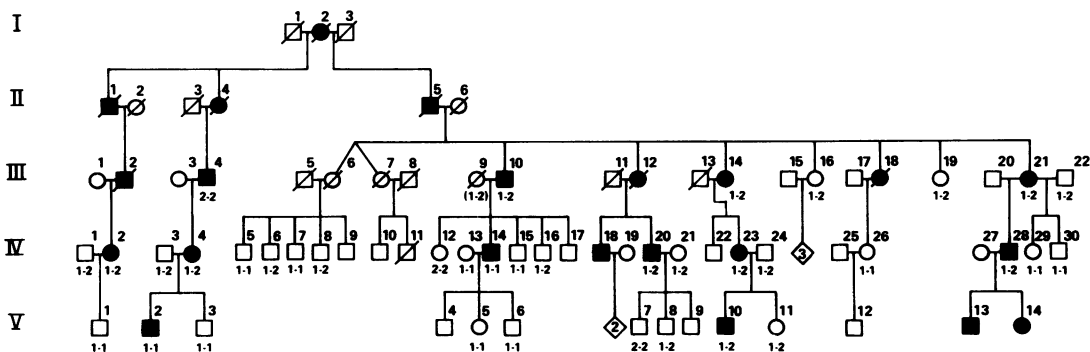


Fig. 2 Dominantly inherited Ehlers-Danlos II pedigree showing *COL2A1* *Hind* III genotypes. Solid figures denote affected individuals and open figures denote unaffected individuals. Squares represent males and circles represent females. Diamonds denote either males or females.

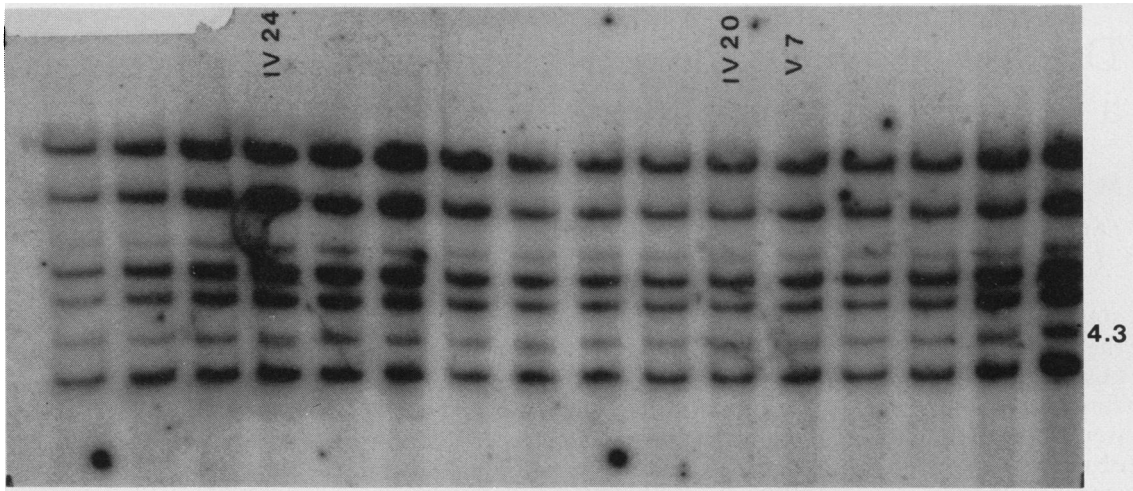


Fig. 3 *EcoRI* digest of 16 members of the kindred. The three numbered tracks demonstrate splitting or widening of the 4.3 kb fragment and are from individuals who are probably heterozygous for length variants beyond the 3' end of the gene.

Asians and West Indians of negro descent.⁸ We suggest that this length polymorphism explains the original report in a West Indian family. Such large deletions are infrequent among Caucasians, but there is still length variation at this locus. Examples are seen in the individuals (IV.20, IV.24, V.7) who are probably heterozygous for alleles differing in length by about 100 bp.

Even though the deletions originally reported were probably normal length variants rather than causal mutations, they could still be useful as markers for the $\alpha_1(\text{II})$ gene. Since in the original small pedigree the EDS gene and the deleted $\alpha_1(\text{II})$ gene were inherited together, it is possible that another mutation elsewhere in the gene could have caused the disease. Alternatively, there is a 25% probability that the cosegregation observed in this small pedigree happened by chance.

Examination of a larger pedigree with a high frequency restriction site polymorphism as a marker for the $\alpha_1(\text{II})$ gene has shown that this gene does not segregate with the EDS gene. We can therefore be certain that the disease, at least in this typical kindred, cannot be caused by a mutation in this gene. Furthermore, the EDS gene cannot be close to the COL2A1 locus on chromosome 12. If EDS II is caused by mutation of a collagen structural gene, then it is far more likely to be a gene encoding a collagen found in skin and ligaments, perhaps type I

or type III, than the major cartilage collagen. We are currently investigating the segregation of other collagen genes in this and similar pedigrees.

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