

A single base mutation in COL5A2 causes Ehlers-Danlos syndrome type II

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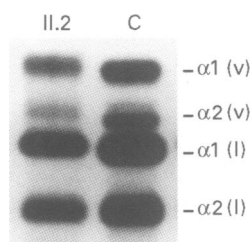


Figure 1 Protein analysis. Radiolabelled proteins secreted from cultured dermal fibroblasts from an affected family member (II.2) and a control (C) were treated with pepsin and subjected to SDS-PAGE and fluorography. The α chains of types I and V collagen were visible as indicated.

Abstract

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of connective tissue disorders. Recently mutations have been found in the genes for type V collagen in a small number of people with the most common forms of EDS, types I and II. Here we characterise a COL5A2 mutation in an EDS II family. Cultured dermal fibroblasts obtained from an affected subject synthesised abnormal type V collagen. Haplotype analysis excluded COL5A1 but was concordant with COL5A2 as the disease locus. The entire open reading frame of the COL5A2 cDNA was directly sequenced and a single base mutation detected. It substituted a glycine residue within the triple helical domain (G934R) of $\alpha 2(V)$ collagen, typical of the dominant negative changes in other collagens, which cause various other inherited connective tissue disorders. All three affected family members possessed the single base change, which was absent in 50 normal chromosomes.

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Mutations in the genes for type V collagen (COL5A1 and COL5A2) have recently been reported¹⁻⁴ in cases of the commonest forms of Ehlers-Danlos syndrome (EDS types I and II). Other EDS types also have defects which result in abnormal collagen molecules. Fibrillar collagens are the most abundant extracellular matrix components and fibrils may be heterotypic, being composed of more than one collagen type.^{5,6} The fibrillar collagen family includes the highly homologous types I, II, III, V, and XI proteins, each with a long uninterrupted triple helix of Gly-X-Y repeats.⁷ Although type V and XI collagens are quantitatively minor fibrillar components, they have an important role in regulating the assembly and structure of the more abundant fibril forming collagens (types I, II, and III), with which they are coexpressed.^{8,9} Type V collagen is more abundant in tissues expressing types I and III collagen, and usually exists as an $[\alpha 1(V)]_2\alpha 2(V)$ heterotrimer,¹⁰⁻¹² which is the product of the COL5A1 and COL5A2 genes. There is evidence that the diameter of fibrils composed of either type I or type II collagen is regulated by the relative proportion of type V or XI collagen respectively. Experiments which artificially altered the ratio of type V or XI col-

lagen to the more abundant collagen types resulted in altered fibril diameters.¹³⁻¹⁵ Also, corneal collagen fibrils, where type V collagen represents a much larger proportion (15-20%) of the total fibrillar collagen,^{16,17} are smaller than in other type I collagen expressing tissues, where type V collagen is less abundant (2-5%).

Not all EDS I/II pedigrees are linked to COL5A1 or COL5A2¹⁸ (N P Burrows, unpublished results) and other candidate genes include those for decorin¹⁹ and tenascin X.²⁰ While it is preferable initially to perform haplotype analysis in multigeneration families, for sporadic cases or small families it is often unhelpful. Here we describe a COL5A2 mutation in a small family which showed the clinical phenotype of EDS II (NPB). Electron microscopic analysis of dermal collagen structure in this family showed only minor variability in the collagen fibril size and shape (data not shown). Cultured skin fibroblasts were labelled with ¹⁴C proline and the collagens synthesised were analysed by SDS polyacrylamide gel electrophoresis (fig 1). Type V collagen from the cell line migrated more slowly than that from a control. Similar observations have been made in cases of osteogenesis imperfecta where mutations in either COL1A1 or COL1A2 cause overmodification of both $\alpha 1(I)$ and $\alpha 2(I)$ collagen proteins.²¹ Thus, in this case it was

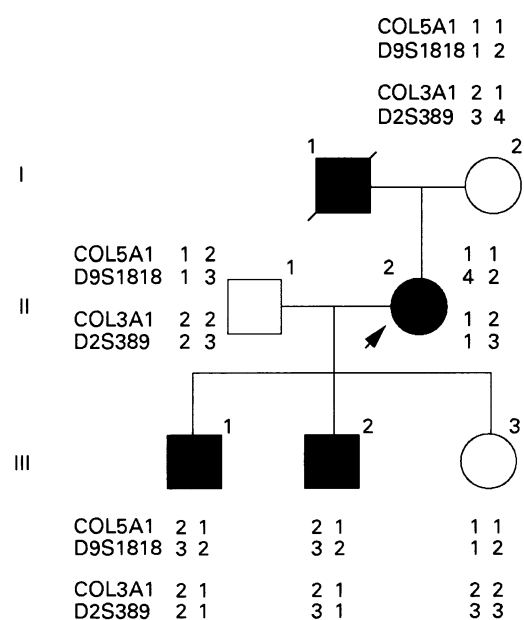


Figure 2 Haplotype analysis. DNA from available family members was amplified using oligonucleotides for various markers as indicated. After separation on a polyacrylamide gel and autoradiography, the haplotype of each subject was scored and is noted on the family pedigree. The arrowhead denotes the proband.

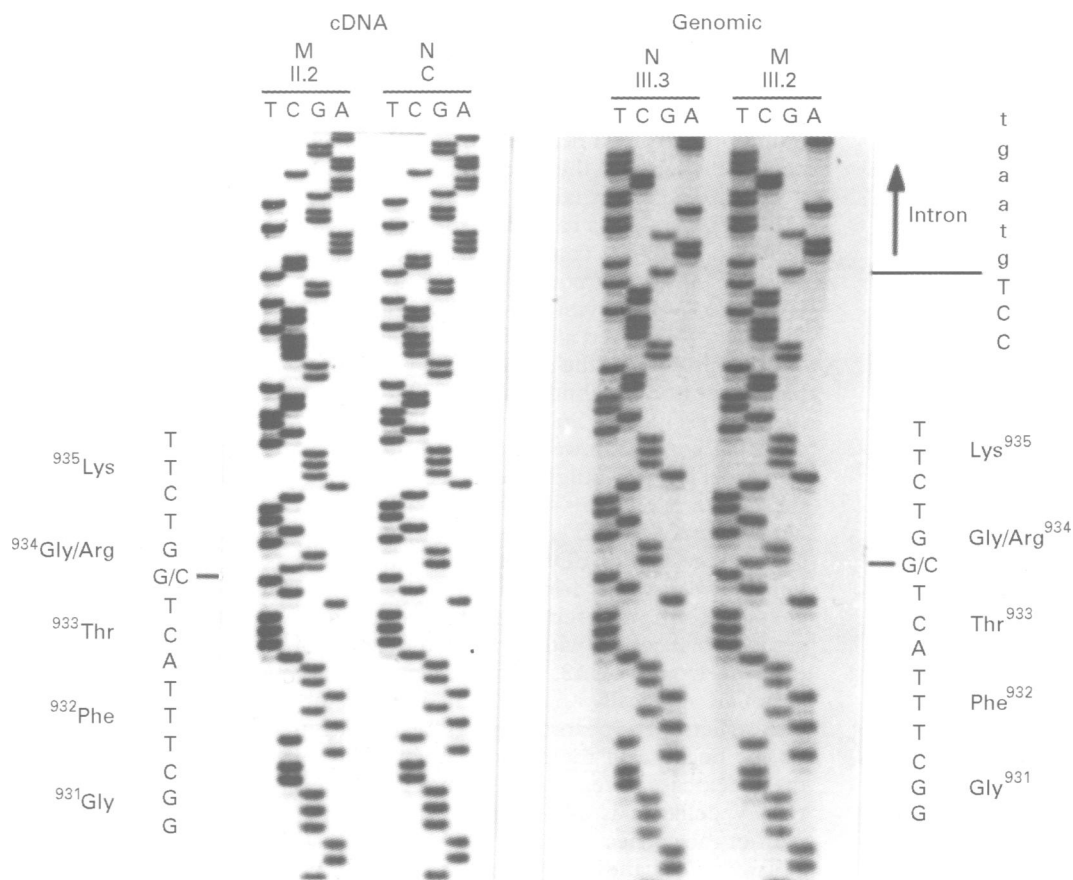


Figure 3 Sequence analysis. The mutant (M) and normal (N) cDNA and genomic sequence obtained from various family members or control (as indicated) is shown. The same sense primer was used for each sequencing reaction. The DNA and corresponding amino acid sequences are written adjacent to the autoradiographs. The heterozygous G/C base change which converts glycine 934 to arginine is indicated, as is the position of the intron within the genomic sequence.

likely that there was a mutation in either COL5A1 or COL5A2.

Haplotype analysis was undertaken using markers (COL5A1, D9S1818, COL3A1, and D2S389) within or close to either COL5A1 or COL5A2.^{18 22-25} The haplotypes (fig 2) excluded COL5A1, but were concordant with COL5A2 as the disease locus. While the family was too small to generate a significant lod score, the combination of protein profile with haplotype analysis strongly implied a causative mutation in COL5A2. The cDNA for the complete coding sequence of $\alpha 2(V)$ collagen (accession NoY14690) was reverse transcribed and amplified from RNA as 11 overlapping products. Each of these were directly sequenced. A single base mutation was seen, which converted an obligatory glycine within the triple helical region of the molecule to arginine (fig 3). This change was confirmed in each affected family member by amplification and sequencing (fig 3) of a 500 bp genomic DNA product. Each affected subject contained the same G to C base change (data are only shown for III.2), and an intron was positioned between bases 3600 and 3601 of the cDNA sequence (fig 3). The mutation abolished a *BsrI* restriction enzyme site which normally removed 80 bp from one end of the 500 bp product. Incubation of the amplified genomic DNA with this enzyme confirmed the sequencing

results (fig 4) and that this change was absent in 50 chromosomes from 25 unrelated controls.

This alteration is typical of many mutations in other fibrillar collagens which cause inherited disorders, such as osteogenesis imperfecta, the chondrodysplasias, and EDS type IV.²⁶ This, along with the absence of this change in normal subjects, provides strong evidence that it is the causative mutation in this EDS II family. Only a few mutations of COL5A1 and

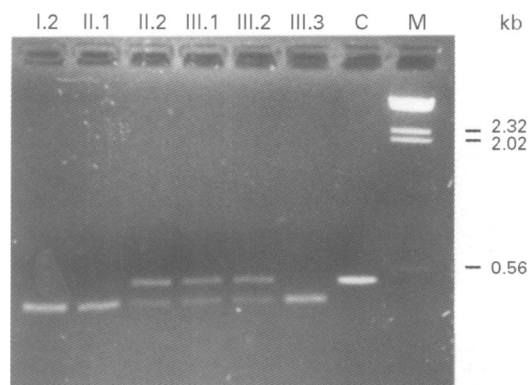


Figure 4 Restriction enzyme digestion. Genomic DNA from each family member (as indicated) was amplified and incubated with the restriction enzyme *BsrI*. Electrophoresis on a 2% agarose gel was used to separate the products, along with undigested DNA (C) and DNA of known sizes used as markers (M).

COL5A2 have been characterised in EDS I/II patients.¹⁻⁴ Three of these affect the C-propeptide and possibly inhibit assembly of the mutant collagen with normal α chains. Others affect the correct splicing of exons coding for part of the triple helix. These, like the mutation described here, will have a dominant negative effect, as they will coassemble with normal collagen α chains.

COL5A2 is also a candidate for Stickler syndrome as $\alpha 2(V)$ coassembles with $\alpha 1(XI)$ collagen in the vitreous²⁷ and a mutation in COL11A1 was previously shown to cause that disorder.²⁸ However, a detailed ophthalmological examination of the proband from this EDS family showed no abnormality of the vitreous, thereby excluding Stickler syndrome. However, the cornea showed keratoconus, a developmental forward bulging of the central area of the cornea. A previous study of 44 patients with keratoconus showed that 50% had clinical signs suggestive of EDS, indicating a possible link between these disorders.²⁹ These corneal changes confirm observations seen in a transgenic mouse model with a targeted deletion of exon 6 from col5a2.³⁰ Heterozygous mice, although clinically normal, had abnormal ultrastructural appearance of corneal collagen fibrils. Because of the corneal abnormality of the index case here, it is likely that in humans type V collagen is also an important component of the cornea.

Two other candidate genes for EDS I/II are those which code for decorin¹⁹ and tenascin X.²⁰ In sporadic cases of EDS I/II, or small families in which exclusion of candidate genes is not possible, variation in clinical signs may provide important clues for determining which gene is mutated. In this instance the index case presented with keratoconus. Unfortunately, as the other two affected subjects were not available for ophthalmic examination, we do not know if it segregates with EDS in this family. However, there has been one previous report of flattened corneas associated with a COL5A1 mutation.² It is possible, therefore, that abnormal corneal morphology may indicate the presence of COL5A1 and COL5A2 mutations in other EDS patients.

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