Am. J. Hum. Genet. 63:390–398, 1998

A Point Mutation in an Intronic Branch Site Results in Aberrant Splicing of COL5A1 and in Ehlers-Danlos Syndrome Type II in Two British Families

Nigel P. Burrows,[1,2,3] Alan C. Nicholls,[1,2] Allan J. Richards,[1,2] Craig Luccarini,[1,2] J. Barrie Harrison,[1,2] John R. W. Yates,[1,4] and F. Michael Pope[1,2,3,4,*]

[1]Department of Pathology, University of Cambridge, [2]Medical Research Council Connective Tissue Genetics Group, Strangeways Research Laboratory, and Departments of [3]Dermatology and [4]Medical Genetics, Addenbrooke's National Health Service Trust, Cambridge

Summary

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of connective-tissue disorders characterized by skin fragility, joint laxity, and skeletal deformities. Type V collagen appears to have a causal role in EDS types I and II, which show phenotypic overlap and may sometimes be allelic. Type V collagen can exist as a heterotrimer, $[\alpha 1(V)]_2\alpha 2(V)$, and it both coassembles with and regulates type I collagen–fibril diameter. Using an intragenic COL5A1 polymorphism, we have demonstrated linkage, at zero recombination, to the same allele in two large British EDS type II families (LOD scores 4.1 and 4.3). Affected members from each family were heterozygous for a point mutation in intron 32 (IVS32:T-25G), causing the 45-bp exon 33 to be lost from the mRNA in ~60% of transcripts from the mutant gene. This mutation lies only 2 bp upstream of a highly conserved adenosine in the consensus branch-site sequence, which is required for lariat formation. Although both families shared the same marker allele, we have been unable to identify a common genealogy. This is the first description of a mutation at the lariat branch site, which plays a pivotal role in the splicing mechanism, in a collagen gene. Very probably, the resulting in-frame exon skip has a dominant-negative effect due to incorporation of the mutant proα chain into the triple-helical molecule. These findings further confirm the importance of type V collagen in the causation of EDS type II, and the novel collagen mutation indicates the importance of the lariat branch site in splicing.

Received September 17, 1997; accepted for publication April 17, 1998; electronically published July 10, 1998.

Address for correspondence and reprints: Dr. Nigel Burrows, Department of Dermatology, Box 46, Addenbrooke's NHS Trust, Hills Road, Cambridge CB2 2QQ, United Kingdom. E-mail: nburrows@hgmp.mrc.ac.uk

*Present affiliation: Medical Research Council Connective Tissue Genetics Group, Institute of Medical Genetics, University Hospital of Wales, Cardiff

0002-9297/98/6301-0014$02.00

Introduction

At least nine clinical subtypes of Ehlers-Danlos syndrome (EDS) are recognized in the Berlin nosology (Beighton et al. 1988). The cardinal manifestations include cutaneous fragility, hyperextensibility, and ligamentous laxity, with occasional skeletal deformities and rupture of hollow organs. All molecular defects in EDS described to date result in abnormalities of the fibrillar collagens (Pope and Burrows 1997). EDS types I (MIM 130000) and II (MIM 130010) show autosomal dominant inheritance, and patients are characterized by atrophic scars, cutaneous hyperextensibility, and joint laxity. EDS type II is less severe than EDS type I, with less propensity to internal organ involvement and premature rupture of fetal membranes. Reported linkage to the COL5A1 gene, which encodes for the $\alpha 1$ chain of type V collagen, in a family with EDS type I and in a mixed-EDS type I/II family, was the first evidence that these disorders can be allelic (Loughlin et al. 1995; Burrows et al. 1996).

Type V collagen is distributed ubiquitously in humans. It coassembles with and regulates fibrillogenesis of type I collagen (Birk et al. 1990; Linsenmayer et al. 1993; Fichard et al. 1995). Type V collagen usually exists as a heterotrimer of pro$\alpha 1$(V) and pro$\alpha 2$(V) chains, encoded by COL5A1 (Greenspan et al. 1992) and COL5A2 (Emanuel et al. 1985), respectively. A third chain (pro$\alpha 3$[V]) exists (Sage and Bornstein 1979), although the COL5A3 gene has not been identified.

A role for type V collagen in EDS was suggested by the identification of an exon-skipping mutation in COL5A1 in a patient with sporadic EDS type II (Nicholls et al. 1994). The presence of short stature and foot abnormalities were also consistent with EDS type VII, but collagen fibril structure, protein chemistry, and DNA analysis excluded this possibility (Nicholls et al. 1996). Several COL5A1 mutations have now been reported, including a translocation through intron 24 in a sporadic EDS type I patient with hypomelanosis of Ito (Toriello et al. 1996), abnormal splicing affecting the C-propeptide in both an EDS type I family (Wenstrup et al. 1996)

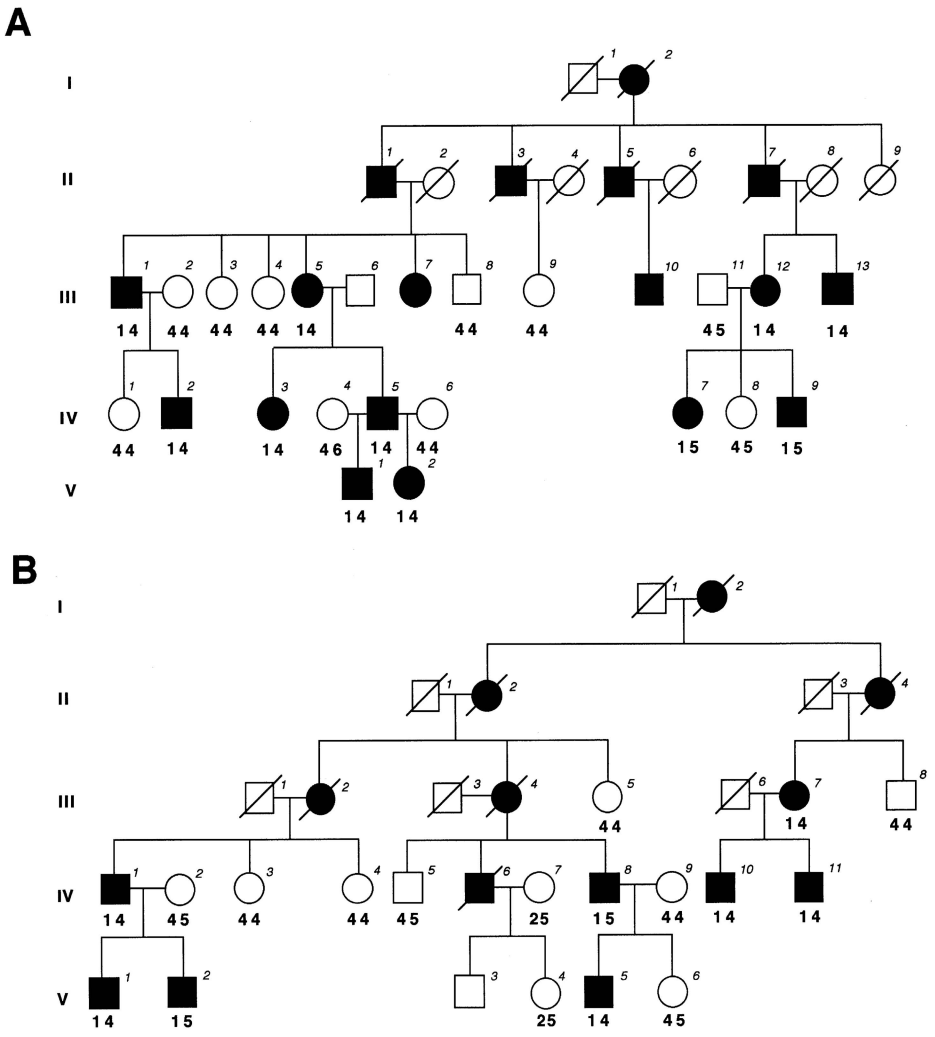

Figure 1 Cosegregation of an intragenic COL5A1 marker with EDS type II phenotype in two families: MK family (*A*) and CH family (*B*). Blackened symbols indicate affected individuals; unblackened symbols indicate unaffected individuals. Allele frequency and size are from Greenspan et al. (1995): 1 (9%, 182 bp); 2 (6%, 188 bp); 4 (69%, 192 bp); 5 (14%, 194 bp); and 6 (1%, 198 bp).

and an EDS type II family (de Paepe et al. 1997), and a cysteine substitution in the C-propeptide in EDS type I (de Paepe et al. 1997). Here we describe a unique collagen mutation of the lariat branch site of COL5A1, resulting in abnormal splicing of exon 33, in two British families.

Material and Methods

This study was approved by the local ethical committee.

Electron Microscopy

Skin biopsy samples were taken, under local anesthetic, from the inner part of the upper arm. One cubic millimeter was processed for electron microscopy as described elsewhere (Nicholls et al. 1996), and the remainder was used for fibroblast culture.

Linkage Analysis

Eleven affected members of the MK pedigree and eight members of the CH pedigree were examined by one of us (N.P.B.). All fulfilled the clinical criteria for EDS type II—namely, atrophic scarring confined to elbows and knees, evidence or history of easy bruising, joint laxity, and moderate cutaneous hyperextensibility. Leukocyte DNA was extracted from 10 ml peripheral blood by means of a Nucleon II kit (Scotlab), in accordance with the manufacturer's instructions. Genotype studies were performed after PCR of a polymorphic simple-sequence repeat (SSR) within intron 17 of COL5A1, as described

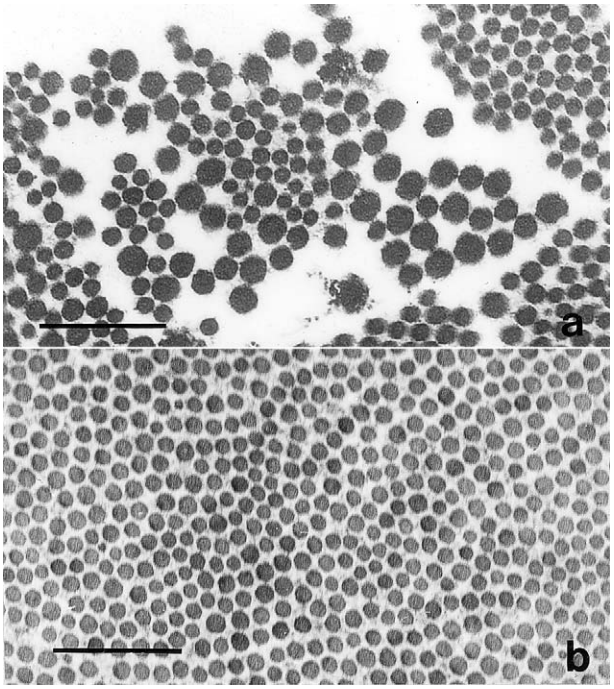

Figure 2 Transmission-electron micrography. Transversely sectioned upper dermal collagen from (*a*) an EDS type II family member (V.5, CH family), showing considerable variation in both size and shape of collagen fibrils (several "cauliflower" fibrils are present), and from (*b*) a clinically unaffected individual from MK family, as a control (IV.8). (Bar = 0.5μm)

elsewhere by Greenspan et al. (1995). Each reaction was performed in 12.5 μl containing a forward primer end-labeled with γ[^{33}P]-ATP and T4 polynucleotide kinase, as described elsewhere (Burrows et al. 1996). Alleles were separated by electrophoresis in a 6% denaturing polyacrylamide gel and were visualized by autoradiography. LOD scores were calculated with the LIPED computer program (Ott 1974), under the assumption of an autosomal dominant disease with a gene frequency of 0.1% and equal male and female recombination fractions. Allele frequencies for the COL5A1 marker were those reported by Greenspan et al. (1995). Three polymorphic markers (CA repeats)—D9S298, D9S114, and D9S1818, which lie in close proximity to COL5A1—were labeled as above. The markers were used for comparison of more-detailed haplotype status of two affected individuals from both families.

cDNA Amplification

Total cytoplasmic RNA was obtained from cultured dermal fibroblasts from at least two affected individuals from both families and was used to reverse transcribe cDNA. Using the cDNA sequence (GenBank, accession number M76729) described by Greenspan et al. (1991) and Takahara et al. (1995), we amplified the cDNA in 17 overlapping fragments of ~300–500 bp in length, to cover the entire open reading frame, as follows: product 1, bases 344–624; product 2, bases 482–924; product 3, bases 854–1287; product 4, bases 1129–1560; product 5, bases 1367–1776; product 6, bases 1679–2067; product 7, bases 1999–2500; product 8, bases 2442–2877; product 9, bases 2822–3163; product 10, bases 3026–3437; product 11, bases 3382–3862; product 12, bases 3757–4177; product 13, bases 4085–4435; product 14, bases 4386–4786; product 15, bases 4726–5201; product 16, bases 5050–5556; and product 17, bases 5342–5803. (Numbers refer to nucleotide positions of the 5′ base of the 20-mer primers.) A reverse primer in exon 33 (bases 2936–2956) was used with a forward primer (bases 854–874) to selectively amplify the non-deleted message. This 2.1-kb fragment was gel purified and reamplified with the same forward and the 1287 reverse primer prior to restriction-enzyme analysis with *Pst*I. Products were electrophoresed in a 4% polyacrylamide gel, stained with ethidium bromide, and visualized under uv light.

Quantitative PCR of fragment 9 was performed by use of a [^{33}P] end-labeled primer (base 3163) for 25 cycles. The product was denatured, run on a 4% de-

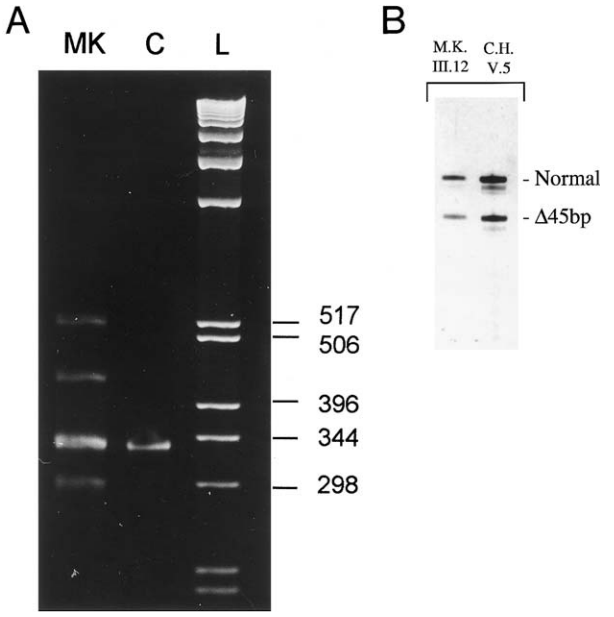

Figure 3 cDNA amplification. *A*, COL5A1 RT-PCR products (2822–3163 bp) from cultured dermal fibroblasts. Affected individual III.12 (MK family) is heterozygous for the deleted product. C = control; L = ladder. *B*, Autoradiograph of a denaturing polyacrylamide gel of RT-PCR products of patients III.12 (MK family) and V.5 (CH family), using a [^{33}P] end-labeled primer for a limited number of cycles. The film was analyzed by densitometry to obtain the relative amounts of normal and deleted transcript species.

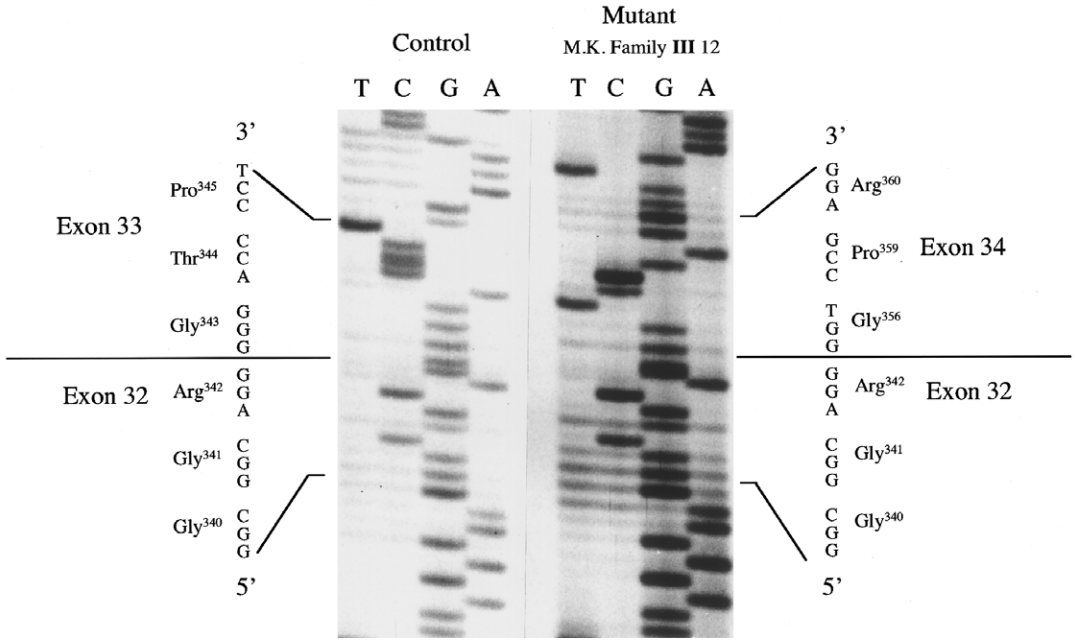

Figure 4 cDNA sequence analysis of sense strand of cloned cDNA showing loss of exon 33 in an affected individual, III.12 (MK family). The nucleotide, amino acid sequences, and exon boundaries are shown. The first glycine of the triple helix has been assigned as number "1."

naturing polyacrylamide gel, and visualized by autoradiography. The band intensities were determined by densitometry, and the ratio of the two transcripts was calculated.

cDNA Sequencing

The normal and shortened cDNA products were separated by electrophoresis in a 2% agarose gel, excised, and eluted in water by means of the Qiaquick gel-elution kit (Qiagen). The cDNAs were made blunt ended with the Klenow fragment of DNA polymerase, phosphorylated with T4 polynucleotide kinase, and then cloned into pBluescript plasmids, in accordance with the manufacturer's instructions (Stratagene). Individual clones were sequenced by use of Sequenase version 2.0 T7 polymerase (U.S. Biochemical) and vector-specific primers, analyzed by electrophoresis in a 6% denaturing polyacrylamide gel, and autoradiographed.

Genomic DNA Analysis

Amplification and sequencing were initially performed to analyze the intron 32 acceptor splice site, exon 33, intron 33, and part of exon 34. The sequence was normal, and, therefore, long-range PCR amplification was performed between exon 32 and exon 34 (~6 kb), by use of Taqplus (Stratagene). Using sequence obtained from this DNA fragment, we designed a forward primer (5′ tgc tct gaa ttc aca gtc tct caa 3′) ~100 bp upstream of the intron 32/exon 33 boundary. This was used with a reverse primer in exon 34 (5′ agt gat gcc ccg ggg gcc tct ttc 3′), for amplification and sequencing reactions. This product was directly sequenced in both directions by means of a Thermo Sequenase cycle sequencing kit (Amersham Life Sciences), and the mutation was identified in both strands. The mutation created an *Alw*I restriction-enzyme site. Genomic DNA from all family members of both pedigrees, 30 normal individuals, and 23 unrelated EDS type I and EDS type II patients was amplified, incubated with *Alw*I restriction enzyme at 37°C overnight, and electrophoresed in 4% polyacrylamide gel prior to being stained with ethidium bromide.

Results

Clinical Findings

Two five-generation British families exhibited similar phenotypes characteristic of EDS type II. All affected individuals demonstrated soft, moderately hyperextensible skin, with scarring confined to elbows and knees, and a variable degree of joint hypermobility. In addition, affected family members had histories of mild to moderate bruising after trauma. There were no varicose veins or skeletal deformities, and there was no history of internal organ involvement or premature births. Individuals III.8 (MK family) and V.4 (CH family) demonstrated extreme joint laxity (Beighton scores [Beighton et al. 1973] 7/9 and 9/9, respectively) but no cutaneous involvement. These features were consistent with benign

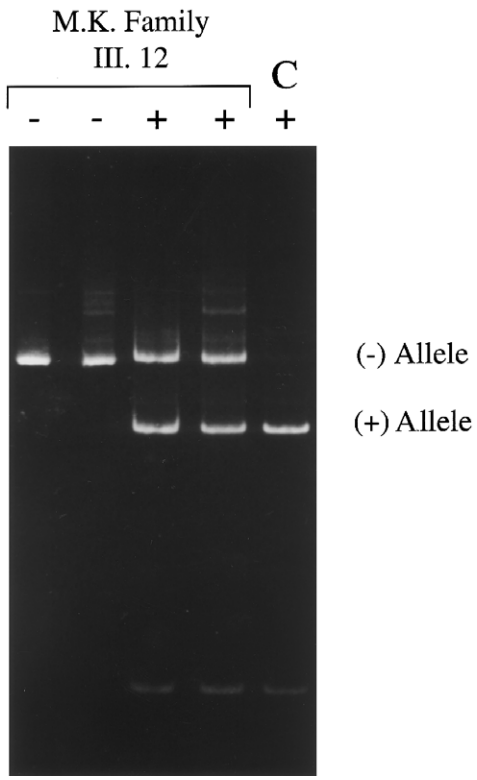

Figure 5 *Pst*I-digestion gel. Ethidium bromide–stained polyacrylamide gel of *Pst*I digests of cDNA PCR amplified specifically from the normal-sized RNA of individual III.12 (MK family) and (C) control (+/+) cDNA. The plus signs (+) and minus signs (−) above lanes indicate whether enzyme was added to the reaction mix.

hypermobile syndrome, and the individuals were, therefore, scored as unaffected.

Ultrastructural Studies

Skin biopsy samples were taken from individuals III.12, III.13, IV.7, IV.8, and IV.9 (MK family) (fig. 1*A*) and from individuals IV.1 and V.5 (CH family) (fig. 1*B*), for dermal fibroblast culture and electron microscopy. Ultrastructural analysis of collagen fibrils in affected individuals consistently showed wide variation in size and shape, in transverse sections (fig. 2*a*). Numerous "cauliflower" fibers were present in three biopsy samples. These features were absent in the biopsy sample from an unaffected individual (IV.8, MK family) (fig. 2*b*).

Molecular Studies

Genomic DNA was obtained from peripheral blood mononuclear cells of affected and relevant unaffected individuals. Analysis of a COL5A1 SSR revealed that allele 1 (frequency 9%, size 182 bp) consistently cosegregated with the EDS type II phenotype, without recombination (fig. 1*A* and *B*). The maximum LOD scores for the MK and CH families were 4.1 and 4.3, respectively, at a recombination fraction of zero, providing significant evidence of linkage of COL5A1 to the EDS type II phenotype. Affected individuals from both families shared a common allele for three polymorphic markers at 9q34 (data not shown).

Mutational analysis of COL5A1 cDNA was performed by use of 17 overlapping reverse transcription–PCR (RT-PCR) products from RNA extracted from cultured dermal fibroblasts. PAGE showed both a normal and a smaller-length product in one fragment (bp 2822–3163) (fig. 3*A*). After denaturation, the deleted product represented ~30% of the total transcript (fig. 3*B*). Cloning and sequencing of the normal and mutant products detected a 45-bp deletion corresponding to exon 33 (fig. 4), which encoded part of the collagen helix (Takahara et al. 1995). After specific amplification of the nondeleted fragment, *Pst*I restriction-enzyme digestion revealed that the cDNA product was heterozygous for this polymorphism, thereby confirming the presence of alternative splicing (fig. 5).

Initial amplification and extensive sequencing of the acceptor splice site at the 3′ end of intron 32, exon 33, intron 33, and part of exon 34 showed the normal sequence. To further analyze the sequence of the 3′ end of intron 32, a 6-kb region, from exon 32 to exon 34, was amplified and partially sequenced. This sequence was used to generate additional primers, which were used to amplify genomic DNA and which showed a T→G point mutation within the branch-site sequence in intron 32 (IVS32:T-25G), 2 bp upstream of the highly conserved adenosine (fig. 6*A* and *B*). This base change created an *Alw*I restriction-enzyme site. PCR products of normal genomic DNA did not contain an *Alw*I restriction-enzyme site, but the presence of the mutation in the PCR product yielded two fragments, of ~230 bp and ~80 bp (fig. 7). Digestion of amplified genomic DNA from all available family members confirmed that the mutation cosegregated consistently with disease, in both families, but was absent in 30 normal individuals and in 23 unrelated EDS type I and EDS type II patients (data not shown).

Discussion

There is convincing evidence that the COL5A1 transversion described above causes EDS type II. First, linkage to COL5A1 is present in both families. Second, the mutation as detected by *Alw*I restriction-enzyme digestion also segregated with the disease phenotype in these families but was absent both from healthy individuals and from other unrelated EDS type I and EDS type II patients. It was therefore unique to these affected families and is not a polymorphism. Third, other COL5A1 mutations occur in EDS types I and II (Nicholls et al. 1996;

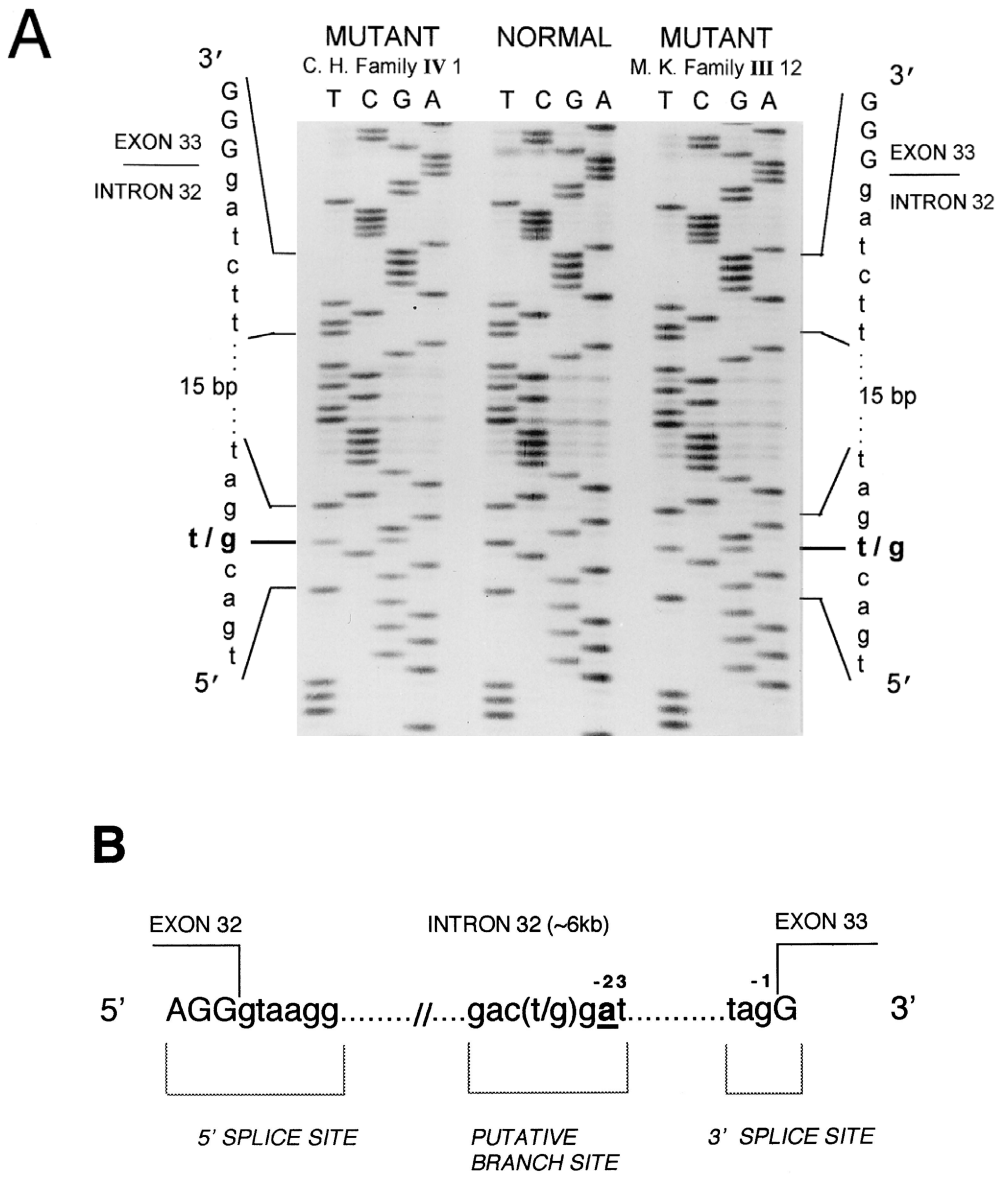

Figure 6 *A*, Genomic DNA sequence analysis, from the junction of intron 32/exon 33, in an EDS type II member from each family (IV.1, CH family, and III.12, MK family) and in a control (normal) individual. The heterozygous T/G base is shown in the affected individuals. *B*, Schematic diagram of mutation. The position of the COL5A1 mutation (IVS32:T-25G) is within the putative branch site. Uppercase letters indicate exon nucleotides; lowercase letters indicate intron nucleotides. The invariant nucleotide of the branch site is underlined, and the mutation is bracketed.

Toriello et al. 1996; Wenstrup et al. 1996; de Paepe et al. 1997), although there have been no reports of a branchpoint mutation, nor are there any previous reports of collagen gene mutations involving this site.

The branch-site consensus region is 6–59 nucleotides upstream of the 3′ splice site (Maquat 1996) and has a weak consensus sequence, Y81 NY100 T87 R81 *A*100 Y94 (Y = pyrimidine; R = purine; N = any base; and numbers represent percentages) (Krainer and Maniatis 1988). We assigned the lariat branch site by identifying the first adenosine 23 bases upstream of the acceptor splice site to be surrounded by sequence homologous to that of the consensus eukaryotic lariat branch site. Adenosine is the single most important nucleotide of branching function and is usually flanked by the sequence UACUAAC, which is the most efficient for mammalian mRNA splicing (Zhuang et al. 1989). It is also the site for binding of the U2 small nuclear ribonuclear protein complex (Reed and Maniatis 1988; Wu and Manley 1989; Query et al. 1994). The mutation in the families

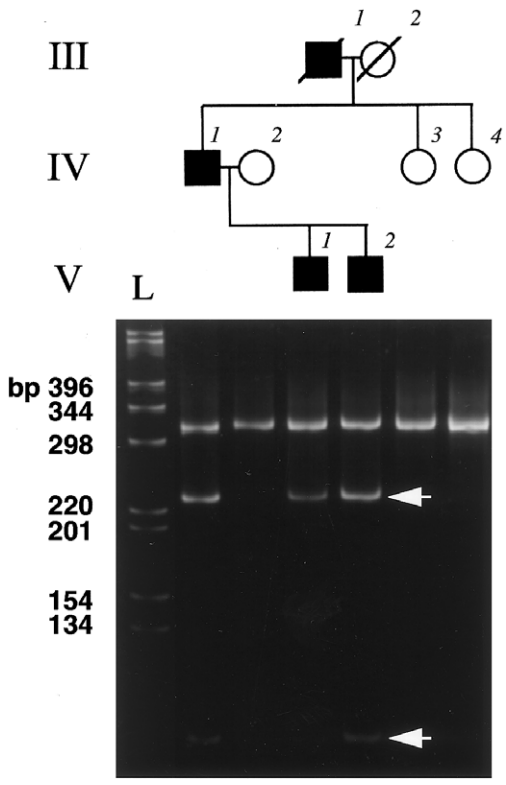

Figure 7 Restriction-enzyme–digestion analysis of genomic DNA from part of the CH family. The same PCR fragment used for genomic sequence analysis was digested with *Alw*I. The products were analyzed by 4% PAGE, along with a 1-kb ladder (L). The extra bands present in the digested DNA from affected individuals are indicated by the arrows.

reported here lies only 2 bp upstream of the highly conserved adenosine. Kuivenhoven et al. (1996) reported a similar branch-site point mutation (IVS4:T-22C) in the lecithin:cholesterol acyltransferase gene, in a family with fish-eye disease, a condition characterized by corneal opacities and low plasma HDL cholesterol. The mutation caused intron retention rather than the exon skipping that occurred in the families we report here. Kuivenhoven et al. (1996) postulated that this occurred because of interference of U2 snRNP binding to premRNA, with failure of correct conformational bulging of the branchpoint adenosine residue essential for the normal branching reaction (Query et al. 1994; Kuivenhoven et al. 1996). Reed and Maniatis (1988) have shown that a U→A base change at the fourth position of the branch site (2 bp upstream of the conserved adenosine) severely reduces splicing efficiency in vitro. Their mutation caused preferential splicing with the normal branch site and 3′ splice site of the downstream intron. In the families reported here, because intron 33 is relatively small (135 bp), its internal branch site could be preferred to the mutated site in intron 32. Consequently, there is "looping out" and loss of the COL5A1 exon 33, which is included in the lariat product. The fact that the deleted product was ~30% of the total suggested that the mutation was allowing alternative splicing of exon 33. To investigate this possibility, we tested several coding-sequence polymorphisms identified during this work, and we found individual III.12 (MK family) to be heterozygous for a *Pst*I polymorphism (at base 967). By specific amplification of the nondeleted cDNA product, we showed that products from both alleles were present, confirming alternative splicing.

Biochemical analyses of collagen synthesized by cultured dermal fibroblasts from affected individuals did not detect any abnormal type V collagen (data not shown). Likewise, de Paepe et al. (1997) previously reported normal migration patterns for type V collagen, despite the presence of abnormally spliced COL5A1 transcripts. However, skipping of exon 33 still leaves the mRNA in-frame but will shorten the helical region of the proα1(V) chain by 15 amino acids. The mutant can still associate with the other proα(V) chains through the functional C-propeptide and thereby can act as a dominant-negative mutation (Marchant et al 1996). We have been unable to determine whether the phenotype is due to decreased amounts of type V collagen, the presence of the mutant molecule, or both.

Type V collagen is found in a large number of diverse tissues, including skin, fetal membranes, bone, cartilage, cornea, and blood-vessel walls. It coassembles with collagen I and III in noncartilagenous tissues (Birk et al. 1990; Linsenmayer et al. 1993). Although less abundant than the other fibrillar collagens, it participates in fibrillogenesis, with a predominantly regulatory rather than structural function (Linsenmayer et al. 1993; Andrikopoulos et al. 1995; Marchant et al. 1996). Diminished type V collagen levels in chick corneas cause abnormally large heterotypic type I/V fibrils because of loss of regulation (Birk et al. 1990; Marchant et al. 1996). It has been suggested that the amino-terminal domains of type V collagen, which are located at the fibril surface, inhibit fibril growth, by steric or electrostatic mechanisms (Linsenmayer et al. 1993; Marchant et al 1996). The ultrastructural abnormalities of dermal collagen in biopsies from these families further support an important regulatory role of type V collagen in matrix assembly.

A mutation involving the branch site is rare and has not been reported previously in any of the collagen genes. Although both of the families we report share the same COL5A1 marker allele, which is found in only 9% of the population (Greenspan et al. 1995), we have been unable to demonstrate a common genealogy. However, using markers in close proximity to COL5A1, we determined that both families shared a number of alleles, suggesting a common founder effect. Furthermore, since

this mutation is not present in 23 other British EDS type I and EDS type II families, it is likely that the two families described herein are distantly related.

Acknowledgments

We are indebted to members of both families for their helpful cooperation in this study. We thank Mrs. M. Laidlaw for maintaining the fibroblast cultures. N.P.B. was in receipt of Wellcome Trust Research Training Fellowship 044116.

Electronic-Database Information

Accession numbers and URLs for data presented in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank
Online Mendelian inheritance in man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for EDS types I [MIM 130000] and II [MIM 130010])

References

Andrikopoulos K, Liu X, Keene DR, Jaenisch R, Ramirez F (1995) Targeted mutation in the *col5a2* gene reveals a regulatory role for type V collagen during matrix assembly. Nat Genet 9:31–36

Beighton P, de Paepe A, Danks D, Finidori G, Gedde-Dahl T, Goodman R, Hall JG, et al (1988) International nosology of heritable disorders of connective tissue, Berlin, 1986. Am J Med Genet 29:581–584

Beighton P, Solomon L, Soskolne CL (1973) Articular mobility in an African population. Ann Rheum Dis 32:413–418

Birk DE, Fitch JM, Babiarz JP, Doane KJ, Linsenmayer TF (1990) Collagen fibrillogenesis *in vitro:* interaction of types I and V collagen regulates fibril diameter. J Cell Sci 95: 649–657

Burrows NP, Nicholls AC, Yates JRW, Gatward G, Sarathachandra P, Richards A, Pope FM (1996) The gene encoding collagen α1(V) (COL5A1) is linked to mixed Ehlers-Danlos syndrome type I/II. J Invest Dermatol 106:1273–1276

de Paepe A, Nuytinck L, Hausser I, Anton-Lamprecht I, Naeyaert J-M (1997) Mutations in the COL5A1 gene are causal in the Ehlers-Danlos syndromes I and II. Am J Hum Genet 60:547–554

Emanuel BS, Cannizzaro LA, Seyer JM, Myers JC (1985) Human α1(III) and α2(V) procollagen genes are located on the long arm of chromosome 2. Proc Natl Acad Sci USA 82: 3385–3389

Fichard A, Kleman J-P, Ruggiero F (1995) Another look at collagen V and XI molecules. Matrix Biol 14:515–531

Greenspan DS, Byers MG, Eddy RL, Cheng W, Jani-Sait S, Shows TB (1992) Human collagen gene COL5A1 maps to the q34.2→q34.3 region of chromosome 9, near the locus for nail-patella syndrome. Genomics 12:836–837

Greenspan DS, Cheng W, Hoffman GG (1991) The pro-α1(V) chain. J Biol Chem 266:24727–24733

Greenspan DS, Northrup H, Au K-S, McAllister KA, Francomano CA, Wenstrup RJ, Marchuk DA, et al (1995) COL5A1: fine genetic mapping and exclusion as candidate gene in families with nail-patella syndrome, tuberose sclerosis 1, hereditary hemorrhagic telangiectasia and Ehlers-Danlos syndrome type II. Genomics 25:737–739

Krainer AR, Maniatis T (1988) RNA splicing. In: Hames BD, Glover DM (eds) Transcription and splicing. IRL Press, Oxford, pp 131–206

Kuivenhoven JA, Weibusch H, Pritchard PH, Funke H, Benne R, Assmann G, Kastelein JJP (1996) An intronic mutation in a lariat branchpoint sequence is a direct cause of an inherited human disorder (fish-eye disease). J Clin Invest 98: 358–364

Linsenmayer TF, Gibney E, Igoe F, Gordon MK, Fitch JM, Fessler LI, Birk DE (1993) Type V collagen: molecular structure and fibrillar organization of chicken α1(V) NH2-terminal domain, a putative regulator of corneal fibrillogenesis. J Cell Biol 121:1181–1189

Loughlin J, Irven C, Hardwick LJ, Butcher S, Walsh S, Wordsworth P, Sykes B (1995) Linkage of the gene that encodes the α1 chain of type collagen (COL5A1) to type II Ehlers-Danlos syndrome (EDS II). Hum Mol Genet 4:1649–1651

Maquat LE (1996) Defects in RNA splicing and the consequence of shortened translational reading frames. Am J Hum Genet 59:279–286

Marchant JK, Hahn RA, Linsenmayer TF, Birk DE (1996) Reduction of type V collagen using a dominant-negative strategy alters the regulation of fibrillogenesis and results in the loss of corneal-specific morphology. J Cell Biol 135: 1415–1426

Nicholls AC, McCarron SM, Narcisi P, Pope FM (1994) Molecular abnormalities of type V collagen in Ehlers-Danlos syndrome. Am J Hum Genet Suppl 55:A233

Nicholls AC, Oliver JE, McCarron S, Harrison JB, Greenspan DS, Pope FM (1996) An exon skipping mutation of a type V collagen gene (COL5A1) in Ehlers-Danlos syndrome. J Med Genet 33:940–946

Ott J (1974) Estimation of the recombination fraction in human pedigrees: efficient computation of the likelihood for human linkage studies. Am J Hum Genet 26:588–597

Pope FM, Burrows NP (1997) Ehlers-Danlos syndrome has varied molecular mechanisms. J Med Genet 34:400–410

Query CC, Moore MJ, Sharp PA (1994) Branch nucleophile selection in pre-mRNA splicing: evidence for the bulged duplex model. Genes Dev 8:587–597

Reed R, Maniatis T (1988) The role of the mammalian branchpoint sequence in pre-mRNA splicing. Genes Dev 2: 1268–1276

Sage H, Bornstein P (1979) Characterization of a novel collagen chain in human placenta and its relation to AB collagen. Biochemistry 18:3815–3822

Takahara K, Hoffman GG, Greenspan DS (1995) Complete structural organization of the human α1(V) collagen gene (COL5A1): divergence from the conserved organization of other characterized collagen genes. Genomics 29:588–597

Toriello HV, Glover TW, Takahara K, Byers PH, Miller DE, Higgins JV, Greenspan DS (1996) A translocation interrupts the *COL5A1* gene in a patient with Ehlers-Danlos syndrome and hypomelanosis of Ito. Nat Genet 13:361–365

Wenstrup RJ, Langland GT, Willing MC, D'Souza VN, Cole

WG (1996) A splice-junction mutation in the region of *COL5A1* that codes for the carboxyl propeptide of the proα1(V) chains results in the *gravis* form of the Ehlers-Danlos syndrome type I. Hum Mol Genet 5:1733–1736

Wu J, Manley JL (1989) Mammalian pre-mRNA branch site selection by U2 snRNP involves base pairing. Genes Dev 3: 1553–1561

Zhuang Y, Goldstein AM, Weiner AM (1989) UACUAAC is the preferred branch site for mammalian mRNA splicing. Proc Natl Acad Sci USA 86:2752–2756