Mutations in the COL5A1 Gene Are Causal in the Ehlers-Danlos Syndromes I and II

Anne De Paepe,[1] Lieve Nuytinck,[1] Ingrid Hausser,[3] Ingrun Anton-Lamprecht,[3] and Jean-Marie Naeyaert[2]

Departments of [1]Medical Genetics and [2]Dermatology, University Hospital Gent, Gent; and [3]Institut für Ultrastrukturforschung der Haut, Universitäts-Hautklinik Heidelberg, Heidelberg

Summary

The Ehlers-Danlos syndrome (EDS) is a heterogeneous connective-tissue disorder of which at least nine subtypes are recognized. Considerable clinical overlap exists between the EDS I and II subtypes, suggesting that both are allelic disorders. Recent evidence based on linkage and transgenic mice studies suggest that collagen V is causally involved in human EDS. Collagen V forms heterotypic fibrils with collagen I in many tissues and plays an important role in collagen I fibrillogenesis. We have identified a mutation in COL5A1, the gene encoding the proα1(V) collagen chain, segregating with EDS I in a four-generation family. The mutation causes the substitution of the most 5′ cysteine residue by a serine within a highly conserved sequence of the proα1(V) C-propeptide domain and causes reduction of collagen V by preventing incorporation of the mutant proα1(V) chains in the collagen V trimers. In addition, we have detected splicing defects in the COL5A1 gene in a patient with EDS I and in a family with EDS II. These findings confirm the causal role of collagen V in at least a subgroup of EDS I, prove that EDS I and II are allelic conditions, and represent a, so far, unique example of a human collagen disorder caused by substitution of a highly conserved cysteine residue in the C-propeptide domain of a fibrillar collagen.

Introduction

The Ehlers-Danlos syndrome (EDS) is a heterogeneous connective-tissue disorder affecting primarily the skin, ligaments, joints, and blood vessels (Steinmann et al. 1993). Several EDS subtypes are caused by mutations in the structural genes for collagen I (EDS VII A and B) and III (EDS IV) or in genes coding for enzymes involved in collagen I biosynthesis (EDS VI and VIIC) (Steinmann et al. 1993). Abnormalities in size and structure of dermal collagen fibrils are found also in EDS I, II, and III (Holbrook and Byers 1982; Hausser and Anton-Lamprecht 1994). The genes encoding the major fibrillar collagens I, II, and III have been excluded as the mutant loci for EDS II (Wordsworth et al. 1985, 1991).

A confident clinical diagnosis of special EDS types is often quite difficult: the intra- and interfamilial variation in expression of clinical symptoms, as well as the influence of genetic background and epigenetic factors, i.e., age-dependent alteration is remarkable; moreover, the assessment of clinical severity is inevitably a matter of interpretation and experience. Thus, the unequivocal labeling of a connective-tissue disorder presenting as EDS by a clear molecular genetic defect is an important contribution to the efforts in genetic counseling, prognosis, and therapeutic management.

Growing evidence suggests that another fibrillar collagen, type V, is causally involved in EDS. Collagen V is coexpressed with collagen I in many tissues and plays an important role in collagen I fibrillogenesis (Birk et al. 1990; Linsenmayer et al. 1993). It consists of three different polypeptide chains, i.e., proα1(V), proα2(V), and proα3(V), encoded, respectively, by the COL5A1 (9q34.2-q34.3; Greenspan et al. 1992), COL5A2 (2q31; Emanuel et al. 1985), and COL5A3 gene (not mapped). Linkage studies performed in three families with EDS II (Loughlin et al. 1995) and in one family with EDS I/II (Burrows et al. 1996) have shown linkage to the COL5A1 gene. On the other hand, Greenspan et al. (1995) have excluded this gene as the causal locus in a large EDS II family. In addition, it has been shown that transgenic mice homozygous for an exon 6 deletion in the COL5A2 gene have phenotypic features reminiscent of human EDS (Andrikopoulos et al. 1995). Recently, evidence for the causal involvement of COL5A1 in EDS I was given by Toriello et al. (1996), who characterized a translocation disrupting the COL5A1 gene in a patient with EDS I and hypomelanosis of Ito. In addition, Wenstrup et al. (1996) have characterized a splicing abnormality causing the skipping of the COL5A1 exon 65 in a family with EDS I.

Received July 22, 1996; accepted for publication December 23, 1996.

Address for correspondence and reprints: Dr. A. De Paepe, Department Medical Genetics, University Hospital Gent, De Pintelaan 185, B-9000 Gent, Belgium. E-mail: anne.depaepe@rug.ac.be

0002-9297/97/6003-0010$02.00

Here, we report the characterization of a mutation in the COL5A1 gene in a four-generation family with EDS I, which substitutes a highly conserved cysteine residue within the proα1(V) C-propeptide domain with serine. In addition, we have identified splicing abnormalities in the COL5A1 gene in two other unrelated families with EDS I or II.

Subjects and Methods

Subjects

Family 1.—The index patient (III-2 in pedigree; see fig. 1*A*) is a 40-year-old man. He was born prematurely with unilateral clubfoot and inguinal hernia. He presented skin and joint hyperextensibility, poor wound healing, and easy bruising, since childhood. He showed facial characteristics of EDS I, including extensively wrinkled forehead with several scars, small eyes, mild ptosis, excessive folding of the eyelids, and a short nose with upturned nosetip. He presented several scars on elbows, knees, and shins and had hyperlaxity of all joints, with a Beighton score for hyperlaxity of 9/9 (Beighton et al. 1969). He also had pectus excavatum, mild scoliosis, varicosities of the distal parts of the legs, and typically broad feet with low plantar arches.

Several other family members presented a similar phenotype, including his mother (II-2) who on clinical examination at age 60 years, showed mild skin hyperextensibility, several atrophic scars on elbows, knees and lower extremities, and mild scoliosis. Joint laxity was less pronounced than in her son and involved mainly the small joints. Her skin around the knees was inelastic, showing excessive large folds. Her father (I-3) and aunt (I-1) reportedly showed the same symptoms.

The proband's sister (III-5) also has mild skin and joint hyperlaxity, easy bruising, and scarring, since childhood. One other sister and one brother (III-3 and III-4) are unaffected.

The two eldest children of the proband (IV-1 and IV-2) also showed the typical features of EDS I. The son (IV-1) was born at 32 wk and the daughter (IV-2) at 37 wk of gestation, respectively. The son, aged 17 years, had moderate skin hyperextensibility, generalized joint laxity, and easy bruising and presented several cigarette paper scars and poorly healing wounds on knees and shins. He also had mild pectus excavatum and typical broad flat feet. The daughter, aged 15 years, had pronounced skin hyperextensibility, numerous atrophic scars on the forehead, elbows, and knees, and molluscoid pseudotumors on the shins. She presented with severe generalized joint hypermobility, with a Beighton score of 9/9. She showed thoracic hyperkyphosis and had a facial appearance typical for EDS I, with small eyes and redundant skin on the upper eyelids. The younger son (IV-3) was born at term and had no clinical features of EDS I.

Family 2.—The proband, a 13-year-old girl, presented with classical features of EDS I. She was the only affected in a sibship of five children from healthy nonconsanguineous parents. She was born prematurely after premature rupture of membranes with an umbilical hernia and bilateral dislocation of the hips. She suffered some walking difficulties in the first years of life, because of joint instability.

She had a shoulder dislocation at age 3 years and a luxation of the right knee at age 5 years. On clinical examination, she showed a pale, hyperextensible skin; numerous ecchymoses and paper scars on shins and knees; generalized joint hyperlaxity with genua recurvata; broad, flat feet; epicanthic folds; strabismus; a small, upturned nose; and a deep nasal bridge.

Family 3.—The proband was diagnosed with EDS type II at the age of 23 years. He had a history of easy bruising and abnormal scarring and suffered several luxations of the ankles while playing basketball.

On clinical examination at age 32 years, he presented with moderate skin hyperextensibility and joint laxity and several pigmented paper scars on knees and shins. His two affected sons, aged 9 and 16 years, showed mild skin hyperextensibility, hyperlaxity of several joints, poor wound healing, and several ecchymoses and atrophic scars on the lower legs. None of them had

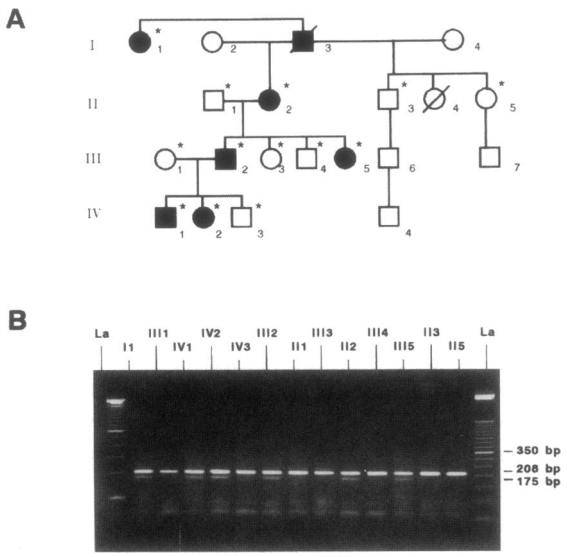

Figure 1 *A*, Pedigree of family 1. The solid black symbols indicate affected family members. Asterisks (*) indicate family members from whom a DNA-sample was analyzed. *B*, *Sty*I restriction pattern found in the different family members from family 1. Genomic DNA was amplified using primers localized within exon 62 of the COL5A1 gene, generating a 208-bp fragment. Affected individuals had an additional *Sty*I restriction site, cleaving the 208-bp fragment into a 175-bp and a 33-bp fragment. A 100- and 50-bp ladder are loaded, respectively, on the left and the right side of the gel.

suffered any major complication of the disease. The overall clinical phenotype was milder than that of the affected patients in family 1 and 2.

Electron Microscopy (Performed only in Family 1)

A knife biopsy was taken from a scar region at the elbow from patient III-2. The skin sample was processed for conventional transmission electron microscopy as reported elsewhere (Hausser and Anton-Lamprecht 1994).

Fibroblast Cultures and Labeling

Skin biopsies used for biochemical analysis were obtained from the proband (III-2 in fig. 1*A*) and his affected mother (II-2 in fig. 1*A*) in family 1, the proband and her parents in family 2, and the proband and his two affected sons in family 3.

Skin fibroblast cultures were established under standard conditions. Labeling of the fibroblasts and purification of the collagen molecules was performed as reported elsewhere (Nuytinck et al. 1996).

Protein Analysis

Collagens were extracted from the medium by alcohol precipitation and redissolved in 0.5 M acetic acid. Medium- and cell-layer samples were counted in a scintillation counter and digested with 50 μg/μl pepsin (Boehringer) for 4 h at 15°C. The digestion was stopped by adding 0.5 μg/μl pepstatin (Boehringer). SDS-electrophoresis was performed using the Laemmli system (Laemmli 1975). The gels were processed for fluorography or for autoradiography, dried, and exposed to a Hyperfilm MP (Amersham). The autoradiographs were scanned and evaluated using a Phosphorimager (Molecular Dynamics).

Linkage Studies

Linkage studies were performed in family 1 with the COL1A1, COL1A2, and COL3A1 genes as described by Sokolov et al. (1991). Linkage analysis with the COL5A1 gene was done using intragenic polymorphisms described by Greenspan and Pasquinelli (1994) and Greenspan et al. (1995).

cDNA Analysis

Total RNA was prepared from skin fibroblasts with Trizol™ (Life Technologies), and first-strand cDNA was synthesized by Moloney murine leukemia virus transcriptase (Life Technologies). Genomic DNA was extracted from peripheral blood leukocytes by the Qiagen-Blood miniprep kit (Qiagen, Inc.). Oligonucleotide primers were designed based on the cDNA structure of the COL5A1 gene (Greenspan et al. 1991) to obtain 12 overlapping PCR fragments amplifying a part of the N-propeptide, the complete α-helix, and a part of the C-propeptide. PCR products were visualized by ethidium bromide.

Heteroduplex analysis (HA) was performed with MDE™ gels (1 × MDE-gel supplemented with 10% glycerol in 1 × Tris-borate EDTA). The bands were visualized by ethidium bromide and photographed. Fragments showing an abnormal migration pattern after HA analysis were cloned using the pCR-Script (SK+) cloning kit (Stratagene) according to the manufacturer's instructions and sequenced using Auto Read Sequencing Kit and an Automated Laser Fluorescent Sequencer (Pharmacia).

Genomic DNA Analysis

Family 1.—The mutation created a *Sty*I restriction site, which was used for restriction analysis of amplified, genomic DNA corresponding to exon 62 of the COL5A1 gene in all the family members. The digested PCR products were separated on a 2.5% agarose gel. Genomic DNA from 200 unrelated individuals and from 12 other patients with EDS I and EDS II was amplified and digested as described above.

Family 2.—Primers were designed to amplify 80 bp at the 3′ end of IVS 41, the whole exon 42 (108 bp), and 80 bp at the 5′ end of IVS 42. The amplified fragment was cloned and sequenced as described above.

Family 3.—Primers were designed to amplify the branch site, the 3′ splice site, and the 5′ splice site of exon 65. Amplimers were cloned and sequenced as described above. The mutation created a *Bsr*I restriction site. Genomic DNA from the two other affected family members and 30 unrelated controls was screened by *Bsr*I restriction analysis.

Results

Histological and Ultrastructural Studies

By light microscopy, the dermal connective tissue showed an unusually loose organization with many small collagen bundles and prominent elastic fibers. The distribution of the dermal components and cells seemed to be disturbed. Within the reticular dermis, conspicuous large collagen bundles appeared "cloudy" and stained deeply with methylene blue.

By electron microscopy (fig. 2*A*, *B*), most collagen bundles contained numerous aberrant collagen fibrils with extremely varying diameters and flowerlike cross-sections, but also highly irregular contours. The collagen bundles that were aberrant by light-microscopic observation consisted of dense aggregations of bizarre fibrils with pronounced variation in shape and diameter; within central regions of these collagenous masses, single fibrils could hardly be distinguished and identified. According to the classification of EDS I–III by differential ultrastructural aberrations of collagen fibrils (Haus-

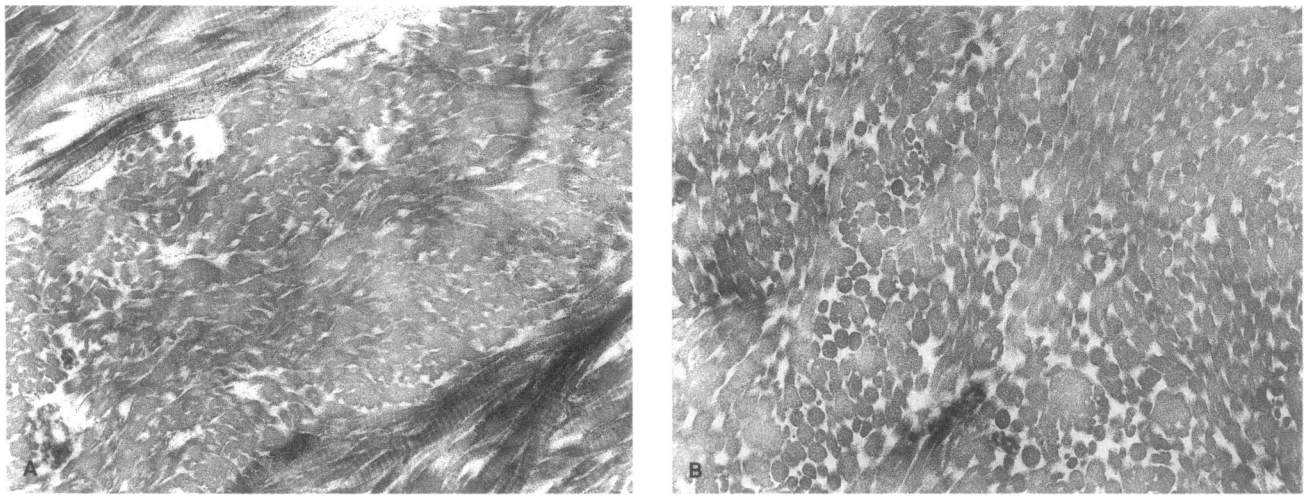

Figure 2 Electron microscopy of a skin biopsy from the proband in family 1, showing chaotically organized collagen bundles in the mid-dermis consisting of densely packed and tangled fibrils with irregular, extremely variable shapes and diameter. *A*, Longitudinal sections, revealing the typical collagen fibril cross-banding periodicity and bizarre-shaped cross-sections. Magnification × 23.000. *B*, Cross-sections. Magnification × 30.000.

ser and Anton-Lamprecht 1994), such collagen material is characteristic for EDS I.

Elastic fibers were fragmented and porous and often contained focal calcifications and were not distributed regularly between collagen bundles, but crowded together. Since connective-tissue components are interactive and consist of interdependent molecules, the morphological alterations of the elastic material and the overall disintegrated dermal organization strengthen the idea of general consequences of a single genetic defect.

Biochemical Collagen Studies

Family 1.—The proband (III-2) and his mother (II-2) showed normal migration patterns for collagen I, III, and V in medium and cell layer. However, the intensity of the α1(V) and α2(V) bands in the cell layer was reduced as compared to a control (fig. 3). Scanning densitometry confirmed that the amount of collagen V proportional to collagen I was decreased by ⩾50% in affected individuals as compared to controls (table 1).

Families 2 and 3.—The affected family members showed normal migration patterns for collagen I, III, and V in medium and cell layer. The intensity of the α1(V) and α2(V) bands in the cell layer appeared slightly decreased compared to the controls.

Molecular Studies

Family 1.—Linkage analysis has excluded the COL1A1, COL1A2, and COL3A1 genes in this family but was inconclusive for the COL5A1 gene (data not shown). HA of the COL5A1 gene revealed a migration shift in the cDNA fragment encoding exons 58–63. Sequencing of the cloned PCR product showed a G-to-C transversion that resulted in the substitution of cysteine (TGC) by serine (TCC) in position 1181 of the proα1(V) collagen chain (fig. 4) (Numbers starting from the first glycine residue in the proα1(V) collagen helix). The mutation created a restriction site for the endonuclease *Sty*I. The presence of the mutation was confirmed in all affected family members by *Sty*I restriction digestion and were shown to be absent in all unaffected family members (fig. 1*B*). The mutation was not found in 200 unrelated healthy individuals nor in 12 unrelated patients with EDS I or II, indicating that this mutation neither represented a common polymorphism nor was the cause of the EDS phenotype in the other patients studied.

Family 2.—RT-PCR amplification performed on cDNA encoding exons 40–46 of the COL5A1 gene showed a fragment of the expected size and a fragment shortened by ~100 bp (fig. 5) because of the deletion of exon 42. Cloning and sequencing of genomic DNA fragments spanning exon 42, the flanking 3′ and 5′ splice sites, and the branch site revealed no sequence abnormalities in these regions. As such, the mutation causing the deletion of exon 42 remains to be defined.

Family 3.—HA of the COL5A1 cDNA revealed several migration shifts in the cDNA fragment encoding exon 63 to a part of exon 66. Sequencing of the corresponding genomic DNA region revealed a T-to-A transversion at position −11 of the 3′ splice site of exon 65. This transversion caused abnormal splicing of the COL5A1 gene yielding two different mRNA products (fig. 6). One mRNA contained a 9-bp insertion because of the use of a new 3′ splice site within IVS 64. The second mRNA contained a deletion of 45 nucleotides at the 3′ end of exon 65, because of the use of a cryptic

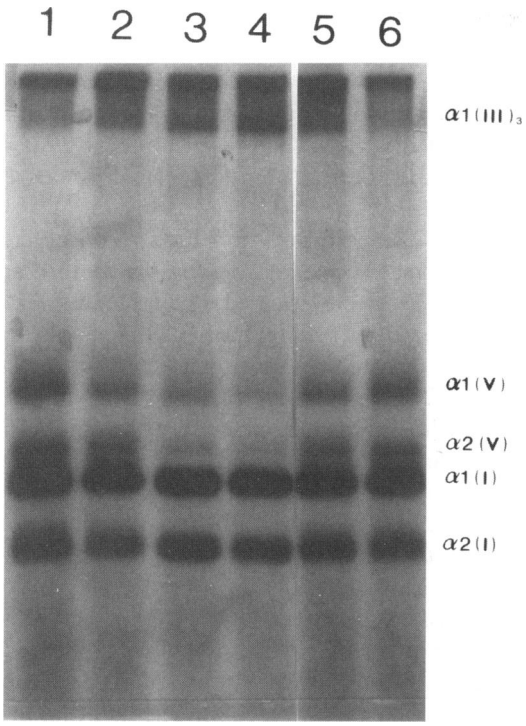

Figure 3 SDS-PAGE of radio-labeled, pepsin-digested collagens from the cell layer derived from family 1. α1(I), α2(I), α1(V), and α2(V) represent the polypeptide chains of collagen type I and V; $[\alpha 1(III)]_3$ represents collagen III homotrimers. The intensity of the bands representing the α1(V) and α2(V) collagen chain is reduced in the EDS I patients (Lane 3, II-2; and Lane 4, II-2) as compared to controls (Lanes 1, 2, 5, and 6).

splice site within exon 65. The mutation created a *Bsr*I restriction site, which allowed us to confirm the presence of the mutation in the other EDS subjects and absence of the mutation in 30 control individuals.

Discussion

In this study, we have identified a mutation in the COL5A1 gene in a four-generation family with EDS I, a sporadic patient with EDS I, and a family with EDS II. The involvement of collagen V in EDS I and II was first shown by linkage studies (Loughlin et al. 1995; Burrows et al. 1996) and subsequently by the discovery of a translocation disrupting the COL5A1 gene in a patient with EDS I (Toriello et al. 1996). Recently, Wenstrup et al. (1996) identified a splicing defect in the COL5A1 gene in a family with EDS I.

Collagen V has been shown to exist in at least two different stoichiometries within tissues (Moradi-Améli

Table 1

Amount of Collagen V Proportional to the Total Amount of Collagen I Plus V Measured by Scanning Densitometry

Lane No.	Sample	Collagen V/Collagen I+V (%)
1	control 1	27
2	control 2	20
3	II2	8
4	III2	8
5	control 1	24
6	control 2	24

NOTE.—In the EDS I patients from family 1, the amount of collagen V is reduced to <50% of the values measured in the controls.

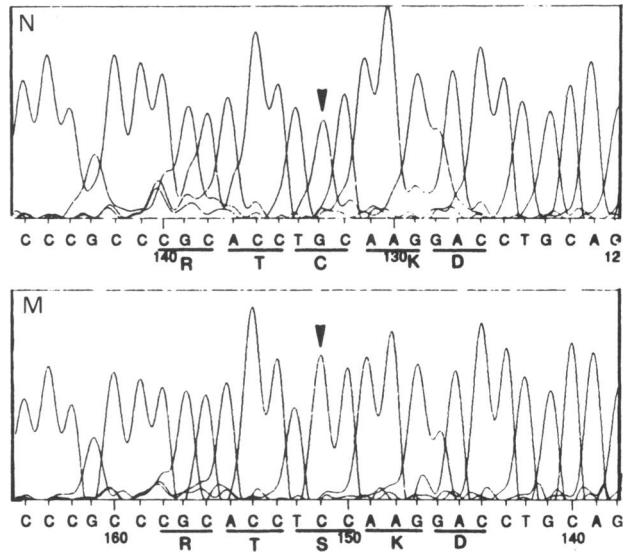

Figure 4 Sequencing data of the normal (N) and the mutant (M) COL5A1 allele in the proband from family 1. A G-to-T transversion changes the codon for cysteine (TGC) in position 1181 of the proα1(V) collagen chain to the codon for serine (TCC).

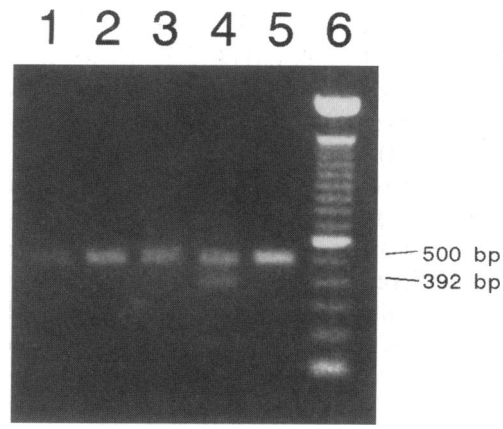

Figure 5 RT-PCR amplification and agarose gel electrophoresis of cDNA encoding exons 40 to 46 in the EDS subject of family 2. Lanes 1–3 and lane 5, control samples. Lane 4, EDS I patient. Lane 6, 100-bp ladder. Separation of the amplimers derived from the patients cDNA revealed a PCR fragment of 500 bp and a second fragment of 392 bp, which showed a deletion of 108 bp.

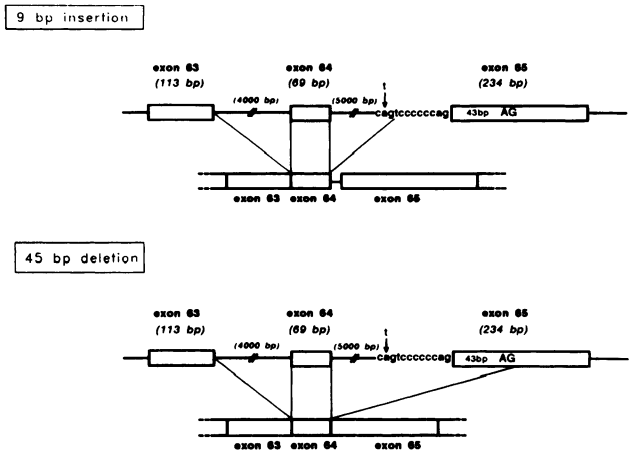

Figure 6 Aberrant splicing of exon 65 of the COL5A1 gene in family 3, caused by the T-to-A transversion in position −11 of the 3′ splice site of IVS 64. The upper panel shows the use of a newly created 3′ splice site within IVS 64, resulting in the insertion of 9 bp derived from IVS 64. The lower panel shows the use of a cryptic splice site within exon 65, resulting in the deletion of the first 45 bp from exon 65.

et al. 1994). The most abundant and widespread population consists of $[\alpha1(V)]_2$ $\alpha2(V)$ heterotrimers in which the N-terminal globular extension of the proα1(V) and proα2(V) chain is retained. It is thought that this N-terminal domain projects onto the surface of the collagen I fibrils as a hingelike region and that it modulates the collagen I fibril diameter by steric hindrance (Linsenmayer et al. 1993). A second population of collagen V molecules found in human tissues consists of $[\alpha1(V)]_3$ homotrimers that are fully processed (Moradi-Améli et al. 1994) and of which the function is not known. A third stoichiometry $\alpha1(V)\alpha2(V)\alpha3(V)$ has been described for placental tissues (Niyibizi et al. 1984) and in postburn granulation tissues (Hashimoto et al. 1988). In addition, heterotypic $(\alpha1[XI])_2$ $\alpha2(V)$ molecules where characterized in bovine vitreous humor (Mayne et al. 1993) and $\alpha1(V)\alpha2(V)\alpha1(XI)$ in bovine bone (Niyibizi et al. 1994).

The mutation reported in family 1 affects the most 5′ of eight conserved cysteines in the C-propeptide domain of the proα1(V) collagen chain. It resides within a region that is highly conserved throughout evolution among the different fibrillar collagens as well as among fibrillar collagens in different species (table 2). This residue is essential for interchain disulphide bonding prior to assembly of the proα-chains and initiation of triple-helix folding of fibrillar collagen molecules (Lees and Bulleid 1994). Most likely, substitution of a conserved cysteine in the proα1(V) carboxyl-propeptide prevents the incorporation of mutant proα1(V) chains into the molecule and results in a reduction of collagen V heterotrimers, as shown by the protein gels and scanning data in the affected members of family 1. In addition, since it has been shown for collagen III that substitution of either one of the 5′ cysteines in the proα1(III) chain prevents interchain disulphide bonding and trimer formation (Lees and Bulleid 1994), the present mutation may also prevent assembly of the homotrimeric form of collagen V.

Although the substitution of a highly conserved cysteine residue in the C-propeptide domain of fibrillar collagens has not yet been reported, several examples of C-propeptide mutations exist in human collagen disorders. In mild forms of osteogenesis imperfecta (OI), deletions in the proα1(I) C-propeptide result in a nonfunctional COL1A1 allele and in a reduction of collagen I (Willing et al. 1994). Similar C-propeptide mutations in collagen X are causal in Schmid metaphyseal chondrodysplasia (McIntosh et al. 1995). In addition, a few mutations in the proα1(I) C-propeptide have also been reported in association with lethal OI (Chessler et al. 1993), although here the disease-causing mechanism is different, since the mutant proα1(I) chains are incorporated and impair helix folding, thereby producing extensively overmodified collagen I molecules. The fact that in family 1 the collagen V molecules were reduced but not overmodified suggests that a mechanism causing functional haploinsufficiency of COL5A1 is involved. This might account also for the numerous aberrant composite or "flowerlike" collagen fibrils within collagen bundles in the upper and deep dermis (as well as in EDS II dermal connective tissue). The prevention of triple helix assembly by lack of interchain disulphide bonding resulting in functional absence of collagen V could well explain the deposition of highly disorganized collagen masses in the mid-dermis characteristic of EDS I.

In family 2, deletion of exon 42 in the COL5A1 gene was found. This exon deletion results in the production of shortened proα1(V) chains. It is possible that these shortened proα1(V) chains are not incorporated in the collagen V trimers or that the trimers containing mutant proα1(V) chains are rapidly degraded intracellularly. This could explain the reduction of collagen V observed in the EDS patient. The underlying genomic mutation in this family is still unknown. A mutation in the sequence spanning exon 42, both splice sites and the branch site, has been excluded. Either the causal mutation is localized in a neighboring region or it remains undetected because of preferential amplification of the normal COL5A1 sequence.

The mutation in family 3 disturbs proper splicing of the COL5A1 gene, respectively, by the use of a newly created 3′ splice site in IVS 64 and by the use of a cryptic splice site in exon 65. As a result, two different proα1(V) chains are produced, one shorter and one longer than the normal proα1(V) chain. It is expected that these mutant proα1(V) chains are not incorporated in the col-

Table 2

Position of the Substituted Cysteine Residue in a Region of the C-Propeptide That Is Highly Conserved throughout Evolution among Fibrillar Collagens in Different Species

Fibrillar Collagen Types in Different Species	Amino Acid Sequence Flanking Conserved Cysteine Residue
α 1(I)—human	K N P A R T C R D L K M C H S D W K S G E Y W I D P N Q G C N
α 1(I)—chick	K N P A R T C R D L K M C H G D W K S G E Y W I D P N Q G C N
α 2(I)—human	K N P A R T C R D L R L S H P E W S S G Y Y W I D P N Q G C T
α 2(I)—chick	K N P A R T C R D L R L S H P E W S S G F Y W I D P N Q G C T
α 1(II)—human	K N P A R T C R D L K L C H P E W K S G D Y W I D P N Q G C T
α 1(II)—chick	K N P A R T C R D I K L C H P E W K S G D Y W I D P N Q G C T
α 1(II)—mouse	K N P A R T C Q D L K L C H P E W K S G D Y W I D P N Q G C T
α 1(III)—human	K N P A R N C R D L K F C H P E L K S G E Y W V D P N Q G C K
α 1(III)—chick	K N P A R N C R D L K F C H P E L K S G E Y W I D P N Q G C K
α 1(III)—rat	K N P A R N C R D L K F C H P E L K S G E Y W V D P N Q G C K
α 1(V)—human	Q N P A R T C K D L Q L C H P D F P D G E Y W V D P N Q G C S
α 1(V)—hamster	Q N P A R T C K D L Q L C H P D F P D G E Y W V D P N Q G C S
α 2(V)—human	K H P A R T C D D L K L C H S A K Q S G E Y W I D P N Q G S C
α 1(XI)—human	T N P A R T C K D L Q L S H P D F P D G E Y W I D P N Q G C S
α 1(XI)—rat	T N P A R T C K D L Q L S H P D F P D G E Y W I D P N Q G C S
α 2(XI)—human	D S P A R T C Q D L K L C H P E L P D G E Y W V D P N Q G C A
α 1(V)—EDS I	Q N P A R T [S] K D L Q L C H P D F P D G E Y W V D P N Q G C S

NOTE.—The cysteine-to-serine substitution in family 1 with EDS I is indicated by a box. The shaded area indicates the region with complete sequence homology.

lagen V trimers because the length difference between mutant and normal proα1(V) chains causes misalignment of their C-propeptide domains prior to chain assembly. The mild reduction in collagen V observed in this family supports this hypothesis.

In conclusion, this paper confirms the causal role of collagen V in at least a subgroup of patients with EDS I or EDS II and demonstrates that both conditions can be allelic. In addition, the important role of the highly conserved cysteine in the C-propeptide domain in fibrillar collagens in chain assembly is illustrated.

Acknowledgments

We are grateful to J. P. Renard, K. Wettinck, and M. Van Thielen for excellent technical assistance. We are indebted to D. Greenspan, University of Wisconsin, for communicating the COL5A1 intron-exon boundary sequences. This work was supported by the National Fund for Scientific Research Belgium.

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, Price A, Lord J, Dickson E (1969) The variants of the Ehlers-Danlos syndrome. Ann Rheum Dis 28:228

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 JRC, 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

Chessler SD, Wallis GA, Byers PH (1993) Mutations in the carboxyl-terminal propeptide of the proα1(I) chain of type I collagen result in defective chain association and produce lethal osteogenesis imperfecta. J Biol Chem 268:18218–18225

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 Nat Acad Sci USA 82: 3385–3389

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 of nail-patella syndrome. Genomics 12:836–837

Greenspan DS, Cheng W, Hoffman GG (1991) The proα(V) collagen chain: complete primary structure, distribution of expression, and comparison with the proα1(XT) collagen 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, tuberous sclerosis I, hereditary hemorrhagic telangiectasia, and Ehlers-Danlos syndrome type II. Genomics 10:737–739

Greenspan DS, Pasquinelli AE (1994) *Bst*UI and *Dpn*II RFLPs at the COL5A1 gene. Hum Mol Genet 3:385

Hashimoto Y, Kobayashi K, Hoshino T, Aoyama H, Hayakawa T (1988) Distinction between two molecular species of type V collagen from human post-burn granulation tissues. J Invest Dermatol 91:238–242

Hausser I, Anton-Lamprecht I (1994) Differential ultrastructural aberrations of collagen fibrils in Ehlers-Danlos syndrome types I–IV as a means of diagnostics and classification. Hum Genet 3:394–407

Holbrook A, Byers PH (1982) Structural abnormalities of the dermal collagen and elastin matrix from the skin of patients with inherited connective tissue disorders. J Invest Dermatol 79:7–16

Laemmli UK (1975) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lees JF, Bulleid NJ (1994) The role of cysteine residues in the folding and association of the COOH-terminal propeptide of types I and III procollagen. J Biol Chem 269:24354–24360

Linsenmayer TF, Gibney E, Igoe F, Gordon MK, Fitch JM, Fessler LI, Birk DE (1993) Type V collagen: molecular structure and fibrillar organization of the chicken α1(V) NH_2-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 V collagen (COL5A1) to type II Ehlers-Danlos syndrome (EDS II). Hum Mol Genet 4:1649–1651

Mayne R, Brewton RG, Mayne PM, Baker JR (1993) Isolation and characterization of the chains of type V/type XI collagen present in bovine vitreous. J Biol Chem 268:9381–9386

McIntosh I, Abbott MH, Francomano CA (1995) Concentration of mutations causing Schmid metaphyseal chondrodysplasia in the C-terminal noncollagenous domain of type X collagen. Hum Mutat 5:121–125

Moradi-Améli M, Rousseau J-C, Kleman J-P, Champliaud M-F, Boutillon M-M, Bernillon J, Wallach J, et al (1994) Diversity in the processing events at the N-terminus of type V collagen. Eur J Biochem 221:987–995

Niyibizi C, Chan R, Wu JJ, Eyre D (1994) A 92 kDa gelatinase (MMP-9) cleavage site in native type V collagen. Biophys Res Commun 202:328–333

Niyibizi C, Fietzek P, van der Rest M (1984) Human placenta type-V collagens: evidence for the existence of an α1(V)α2-(V)α3(V) collagen molecule. J Biol Chem 259:14170–14174

Nuytinck L, Dalgleish R, Spotila L, Renard JP, Van Regemorter N, De Paepe A (1996) Substitution of glycine-661 by serine in the α1(I) and α2(I) chains of type I collagen results in different clinical and biochemical phenotypes. Hum Genet 97:324–329

Sokolov BP, Prytkov AN, Tromp G, Knowlton RG, Prockop DJ (1991) Exclusion of COL1A1, COL1A2 and COL3A1 genes as candidate genes for Ehlers-Danlos syndrome type I in one large family. Hum Genet 88:125–129

Steinmann B, Royce PM, Superti-Furga A (1993) The Ehlers-Danlos syndrome. In Royce PM, Steinmann B (eds) Connective tissue and its heritable disorders, Wiley-Liss, New York, pp 351–407

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

Willing MC, Deschenes SP, Scott DA, Byers PH, Slayton RL, Pitts SH, Arikat H, et al (1994) Osteogenesis imperfecta type I: molecular heterogeneity for COL1A1 null alleles of type I collagen. Am J Hum Genet 55:638–647

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 proα1(V) chains results in the gravis form of the Ehlers-Danlos syndrome (type I). Hum Mol Genet 5:1733–1736

Wordsworth BP, Ogilvie D, Smith R, Sykes B (1985) Exclusion of the α1(II) collagen structural gene as the mutant locus in type II Ehlers-Danlos syndrome. Ann Rheum Dis 44: 431–433

Wordsworth BP, Ogilvie DJ, Sykes BC (1991) Segregation analysis of the structural genes of the major fibrillar collagens provides further evidence of molecular heterogeneity in type II Ehlers-Danlos syndrome. Br J Rheumatol 30: 173–177