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Alu-Alu Recombination Results in a Duplication of Seven Exons in the Lysyl Hydroxylase Gene in a Patient with the Type VI Variant of Ehlers-Danlos Syndrome

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Summary

The type VI variant of the Ehlers-Danlos syndrome (EDS) is a recessively inherited connective-tissue disorder. The characteristic features of the variant are muscular hypotonia, kyphoscoliosis, ocular manifestations, joint hypermobility, skin fragility and hyperextensibility, and other signs of connective-tissue involvement. The biochemical defect in most but not all patients is a deficiency in lysyl hydroxylase activity. Lysyl hydroxylase is an enzyme that catalyzes the formation of hydroxylysine in collagens and other proteins with collagen-like amino acid sequences. We have recently reported an apparently homozygous large-duplication rearrangement in the gene for lysyl hydroxylase, leading to the type VI variant of EDS in two siblings. We now report an identical, apparently homozygous large duplication in an unrelated 49-year-old female originally analyzed by Sussman et al. Our simple-sequence-repeat–polymorphism analysis does not support uniparental isodisomy inheritance for either of the two duplications. Furthermore, we indicate in this study that the duplication in the lysyl hydroxylase gene is caused by an Alu-Alu recombination in both families. Cloning of the junction fragment of the duplication has allowed synthesis of appropriate primers for rapid screening for this rearrangement in other families with the type VI variant of EDS.

Introduction

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of disorders characterized by joint hypermobility, skin fragility and hyperextensibility, and other signs of connective-tissue involvement. The specific features of the type VI variant of the syndrome, which is recessively inherited, are muscular hypotonia, kyphoscoliosis, and ocular manifestations in some patients (Krane 1984; Steinman et al. 1992). In the type VIA variant, the biochemical defect is a deficiency in lysyl hydroxylase activity (Krane et al. 1972; Sussman et al. 1974; Elsas et al. 1978; Krieg et al. 1979; Ihme et al. 1983; Dembure et al. 1984; Chamson et al. 1987; Wenstrup et al. 1989). The patients with the type VIB variant of the syndrome show normal levels of lysyl hydroxylase activity, although the symptoms are similar to those in patients with the VIA variant (Judisch et al. 1976; Ihme et al. 1983; Royce et al. 1989; Wenstrup et al. 1989).

Lysyl hydroxylase (E.C.1.14.11.4) catalyzes the formation of hydroxylysine in collagens and other proteins with collagen-like amino acid sequences. The hydroxylysine residues formed serve as attachment sites for carbohydrates, either galactose or the disaccharide formed from galactose and glucose, and are used to build up collagen cross-links, which are essential for the mechanical strength of connective tissue (see Kivirikko and Myllylä 1980, 1985; Kivirikko et al. 1992).

Molecular cloning of chick (Myllylä et al. 1991) and human (Hautala et al. 1992) lysyl hydroxylase has made it possible to study the mutations leading to the decrease in lysyl hydroxylase activity in the cells of EDS type VI patients. We have recently reported on two mutations leading to the type VI variant of EDS, a homozygous point mutation creating a premature translation stop codon in two siblings (Hyland et al. 1992) and an apparently homozygous large-duplication rearrangement in the gene for lysyl hydroxylase in another two siblings (Hautala et al. 1993). We now report an identical large duplication in a 49-year-old Caucasian female with typical symptoms of EDS type VI (Sussman et al. 1974). Lysyl hydroxylase activity measured in skin fibroblasts of this patient was ~10% of that in the control (Sussman et al. 1974). We now also report that both the duplication in this patient and that in the patients studied elsewhere (Hautala et al. 1993) are caused by homologous recombination of two identical 44-nucleotide regions of Alu sequences in introns 9 and 16 in the gene for lysyl hydroxylase. Our data also suggest that uniparental isodisomy does not explain the homozygosity of the mutation in either of the two families.

Material and Methods

Cell Culture

Fibroblast cultures were established from skin biopsies from a 49-year-old Caucasian woman who was considered

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ered to have EDS type VI (Sussman et al. 1974). Lysyl hydroxylase activity measured from the cells was reported to be ~10% of the activity found in control cells (Sussman et al. 1974). The cells were deposited at the American Type Culture Collection, Rockville, MD (CRL 1195). GM01790 cells, which are from our previously published patient (Hautala et al. 1993), were purchased from the Coriell Institute, Camden, NJ. These cells and locally established human skin fibroblasts were cultured in Dulbecco's modified Eagle's medium (GIBCO) containing 10% FCS. Confluent cells were washed carefully with 0.14 M NaCl in 20 mM phosphate buffer pH 7.4 and were stored at −70°C until analyzed.

Northern and Southern Analyses

Total RNA was isolated from the cells by using the guanidine isothiocyanate method (Ausubel et al. 1989; Sambrook et al. 1989). For northern analysis, the RNA was electrophoresed in a 0.7% agarose gel containing 2 M formaldehyde and was transferred to a nitrocellulose filter that was hybridized at 42°C overnight with a radioactively labeled full-length human lysyl hydroxylase cDNA probe (LH31 in Hautala et al. 1992), under conditions of 50% (v/v) formamide, 5 × SSC (1 × SSC = 0.15 M NaCl, 15 mM sodium citrate buffer, pH 6.8), 1 % (w/v) BSA, 1% (w/v) polyvinylpyrrolidone, 0.250 mg denatured salmon sperm DNA/ml, 0.1% (w/v) SDS. The filters were washed with 0.5 × SSC, 0.1% (w/v) SDS at 55°C.

Genomic DNA was prepared as described elsewhere (Sambrook et al. 1989). Ten micrograms digested DNA was separated on a 0.8% agarose gel and was transferred to a nitrocellulose filter, and hybridization was carried out with the radioactively labeled LH31 cDNA clone (see above). For fingerprint analysis of the DNA from CRL 1195 and GM01790 cells (Hautala et al. 1993), the DNA was digested with *Hin*fI and *Alu*I and hybridized to multilocus probes 33.6 and 33.15 (Jeffreys et al. 1985). The hybridization was carried out in the presence of 6 × SSC, 0.5% milk powder at 62°C for 24 h and was washed under stringent conditions.

cDNA Synthesis, PCR Amplification, and Sequence Analysis

cDNA was synthesized by Moloney murine leukemia virus reverse transcriptase by using total RNA as a template and lysyl hydroxylase–specific oligonucleotides as primers, as described elsewhere (Hautala et al. 1993). The PCR amplification was performed with a Perkin Elmer Cetus thermal cycler by using *Taq* DNA polymerase (Promega) in the presence of 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin. The amplification conditions for cDNA were as described elsewhere (Hautala et al. 1993). Genomic DNA was amplified using a 5′–TTTCCTGTCTCCAGACC–3′ oligonucleotide (JH44) from intron 9 of the lysyl hydroxylase gene and a 5′–CAGTTTTACAACATTCATATC–3′ oligonucleotide (JH71) from intron 16 of the gene as primers. The reaction mixtures were denatured at 94°C for 1 min and were annealed at 56°C, and the extension was carried out at 72°C for 1 min. The annealing temperature was decreased by 2°C every two cycles for three times and was maintained at 50°C for the final 30 cycles. Genomic DNA amplification was carried out under similar conditions by using primer JH55, a 5′–GATCACGAGGTCAGGAGAT–3′ sequence from intron 9, and primer JH 71 (see above).

PCR fragments were sequenced by direct sequencing with the dideoxynucleotide chain-termination method using either T7 DNA polymerase (Sequenase; United States Biochemical) or ΔTaq version 2.0 DNA polymerase with a Perkin Elmer Cetus thermal cycler (*Taq* cycle sequencing kit; United States Biochemical). The sequencing primers were specific 17-mer oligonucleotides for lysyl hydroxylase exon or intron sequences. Sequencing of the duplication junction was carried out after subcloning of the PCR fragment into a TA cloning vector.

PCR-based simple-sequence-repeat–polymorphism analysis was carried out as described elsewhere (Engelstein et al. 1993). The following CA dinucleotide repeat markers were used in the experiments: DIS160, DIS312 (Engelstein et al. 1993), and FGR (Patel et al. 1992). In the amplification, one labeled primer was used for each locus, and the amplification products were separated on a 6% sequencing gel, with a known sequence ladder used as a size reference.

Results

Southern and Northern Analyses

Southern blot analysis was carried out by digesting the genomic DNA from dermal fibroblasts of the patient (ATCC CRL 1195), by using *Eco*RI and *Bam*HI restriction enzymes, and the fragments hybridizing with the cDNA for human lysyl hydroxylase are shown in figure 1. Five fragments were present in the DNA of normal cells after *Bam*HI digestion, whereas six fragments were seen in the proband's cells. The data indicate that there is an additional band in CRL 1195 cells and that the sum of the fragments hybridizing with lysyl hydroxylase cDNA is significantly larger in the proband's cells than in the control cells. The *Bam*HI-digested DNA from the CRL 1195 cells migrated in an anomalous manner, compared with the DNA obtained from a control cell line, and digestion of DNA from several normal cell lines indicates that between different individuals there is variation in the mobility of *Bam*HI restriction fragments (not shown). Our preliminary data suggest a polymorphism at *Bam*HI restriction sites. *Eco*RI digestion produced DNA fragments of 5.2, 6.6, and 13.2 kb in normal cells, whereas a fragment of ~20 kb was seen in addition to the 5.2- and 6.6-kb fragments in the

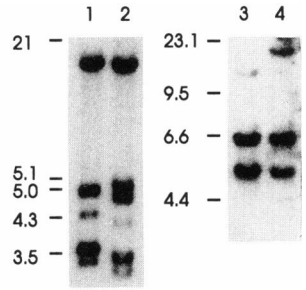
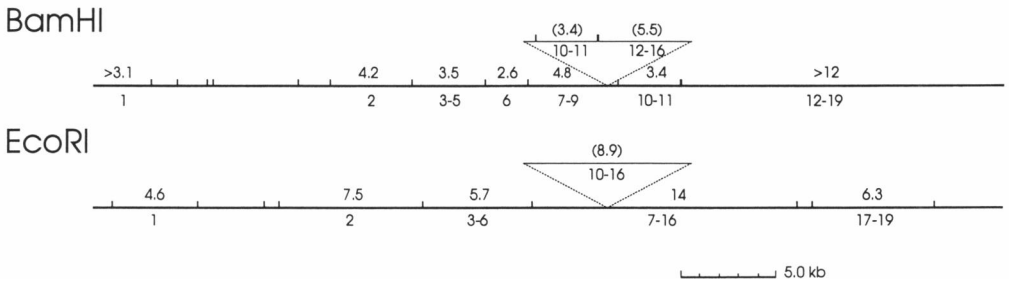


Figure 1 *Right,* Southern analysis of DNA from proband and control cells probed with a radioactively labeled full-length cDNA of lysyl hydroxylase: genomic DNA of control (lane 1) and proband (lane 2) digested with *Bam*HI and of control (lane 3) and proband (lane 4) digested with *Eco*RI. Migration of DNA markers (3.5–23.1 kb) is indicated. *Below,* Restriction map of *Bam*HI and *Eco*RI sites in the lysyl hydroxylase gene. Short vertical lines indicate restriction sites. The numbers above the line indicate the size (in kb) of the fragment, and the numbers below the line indicate the exons present in the fragment. (The exons are numbered from the 5′ end of the gene and are based on the data of J. Heikkinen, T. Hautala, K.I. Kivirikko, and R. Myllylä, unpublished data). The inserted lines represent duplicated DNA fragments present in the CRL 1195 cells, and the numbers in parentheses show the sizes (in kb) of the fragments produced by *Bam*HI and *Eco*RI digestion.



CRL 1195 cells. No band was seen in the Southern blot corresponding to the 13.2-kb fragment found in the *Eco*RI digest of DNA from normal cells, indicating that the allele giving the normal *Eco*RI restriction pattern is not present in the CRL 1195 cells. We have constructed a restriction map (fig. 1, *lower panel*) from *Bam*HI and *Eco*RI restriction sites in the lysyl hydroxylase gene, on the basis of a restriction map of the human lysyl hydroxylase gene structure (authors' unpublished data). *Bam*HI digestion produces seven genomic fragments, and *Eco*RI five genomic fragments, that could be hybridized with lysyl hydroxylase cDNA. In addition, there are fragments produced by *Bam*HI and *Eco*RI that are from intron regions of the gene. Our Southern blot data are in agreement with this restriction map. Except for the *Bam*HI fragment containing exon 2, however, all fragments containing only one exon do not hybridize with the cDNA in our experiment. On the basis of the restriction map and our sequencing data of the lysyl hydroxylase cDNA in CRL 1195 cells (see other results of this study), we estimate that the length of the gene encoding lysyl hydroxylase in the CRL 1195 cells is 8.9 kb larger than the gene in normal cells. Northern-blot data indicate that the mRNA for lysyl hydroxylase produced in the proband's cells was 4 kb in size, whereas the mRNA in normal cells was only 3.2 kb (fig. 2, *left*).

Sequence Analysis

The entire coding region and almost all of the 3′ noncoding region of the lysyl hydroxylase cDNA, constituting ~3,000 nucleotides, was sequenced from skin fibroblasts of the proband. The results of the sequence analysis indicate that the proband has an mRNA for lysyl hydroxylase, in which a region corresponding to amino acids 326–585 in the normal lysyl hydroxylase sequence (Hautala et al. 1992) is duplicated. As a result of this duplication, the lysine at position 585 of the normal sequence is followed by glutamic acid, glutamine, histidine, etc. in positions 326, 327, 328, etc. of the normal sequence. The cDNA region carrying the mutation could be analyzed by amplification (fig. 2, *right*). A fragment of ~800 nucleotides was found when the cDNA of the proband cells was amplified using oligonucleotide primers TH42 and TH38 (Hautala et al. 1993). These primers hybridize to sequences located close to each other in normal cDNA and amplify a DNA fragment <100 nucleotides in size.

In order to be sure that the cell line studied here is not the same that was analyzed earlier (Hautala et al. 1993), a Southern blot hybridization with multilocus probes was carried out (fig. 3). The multilocus probes used in this study, 33.6 and 33.15 (Jeffreys et al. 1985), hybridize to human DNA with several highly variable regions simultaneously, creating a pattern of DNA fragments. The pattern is specific for each individual and consequently is referred to as a "DNA fingerprint." As shown in figure 3, the pattern of DNA fragments from the CRL1195 cells is totally different from that found in the GMO1790 cells (Hautala et al. 1993), indicating that the cells are from different individuals.

Characterization of the Duplication Junction

According to our analysis of the human lysyl hydroxylase gene (J. Heikkinen, T. Hautala, K. I. Kivirikko, and R. Myllylä, unpublished data), the duplicated region corresponds to seven exons, and the rearrangement occurs in the intron region. The DNA of the proband contains a tandem repeat of the region containing exons 10–16. In

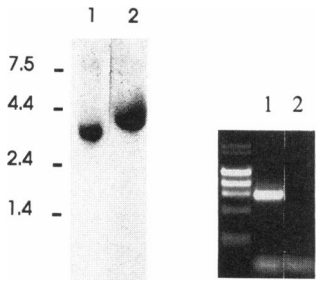


Figure 2 *Left,* Northern analysis of lysyl hydroxylase mRNA in control (lane 1) and proband (lane 2) cells run in the same gel and probed with a radioactively labeled full-length cDNA of lysyl hydroxylase. The migration of molecular-weight markers (1.4–7.5 kb) is indicated. *Right,* Amplification of lysyl hydroxylase cDNA prepared from RNA of proband (lane 1) or control (lane 2) cells. Lysyl hydroxylase–specific oligonucleotides TH38 and TH42 (Hautala et al. 1993) were used for the amplification. These oligonucleotides amplify, in the control cells, a DNA fragment of ~60 nucleotides, which is seen in the front of this agarose gel. The molecular-weight markers (2.3, 2.0, 1.35, 1.08, 0.87, 0.60, and 0.31 kb) are shown on the left.

order to map the exact nucleotide positions of the rearrangement, we sequenced introns 9 and 16 of the normal lysyl hydroxylase gene. Intron 9 consists of 1,987 nucleotides, and intron 16 contains 3,578 nucleotides. Both introns have identical 44-nucleotide sequences, which are located 1,075 and 1,549 nucleotides toward the 3′ direction of the exon-intron boundary in intron 9 and intron 16, respectively (fig. 4, *top*). Amplification of the mutated genomic region by using an oligonucleotide from intron 16 paired with oligonucleotides from intron 9 suggested that the duplication junction is located between oligonucleotide primers JH71 and JH44 (fig. 4, *top*). The distance between these oligonucleotides is ~8 kb in the normal lysyl hydroxylase gene. The use of these primers does not

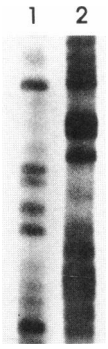


Figure 3 DNA fingerprint analysis of CRL1195 (lane 1) and GMO1790 (lane 2) cells. DNA extracted from the cells was digested with *Hin*fI and *Alu*I, was transferred to a nitrocellulose filter, and was hybridized to radioactively labeled multilocus probes (see Subjects, Material, and Methods).

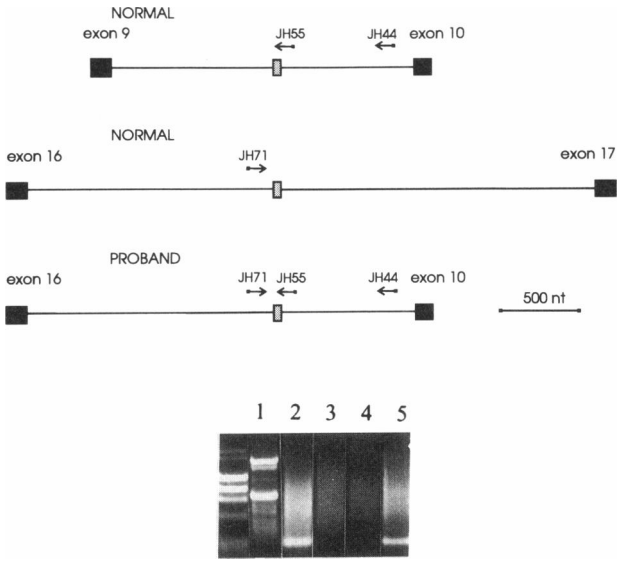


Figure 4 *Top,* Map of the duplication junction (line 3) and corresponding normal introns (lines 1 and 2) in the lysyl hydroxylase gene. The figure shows the location of oligonucleotide primers JH71 (forward primer) and JH55 and JH44 (reverse primers). The shaded box shows the identical 44-nucleotide sequence of the introns. *Bottom,* Amplification of the genomic DNA from the proband's cells (lanes 1 and 2) and from control cells (lanes 3 and 4), by using either oligonucleotide primers JH71 and JH44 (lanes 1 and 3) or oligonucleotide primers JH71 and JH55 (lanes 2 and 4). Sequencing of the DNA fragments in lane 1 indicated that a 960-bp fragment contained the junction of intron 16 and intron 9 (see text). The uppermost bands are nonspecific PCR products. Lane 5 shows the amplification of DNA of GM01790 cells, another cell line with a duplication of seven exons in the lysyl hydroxylase gene (Hautala et al. 1993), by using oligonucleotide primers JH71 and JH55. The molecular-weight markers (2.3, 2.0, 1.35, 1.08, 0.87, 0.60, and 0.31 kb) are shown on the left.

amplify a sequence in the DNA from normal cells, whereas two major fragments are obtained in the amplification of the proband's genome (fig. 4, *bottom*). The sequences of these amplified fragments indicate that the 960-bp fragment contains nucleotide sequences from intron 16 and intron 9. Amplification of the proband's genome by using oligonucleotide primers JH71 and JH55 resulted in a band of ~300 bp corresponding to the duplication junction (fig. 4, *bottom*). Sequencing of this duplication junction indicated that a rearrangement in the lysyl hydroxylase gene has occurred between these identical elements of introns 9 and 16 (fig. 5). No insertions, deletions, or mismatches were observed between the rearrangement junction fragment and the corresponding wild-type regions, suggesting that the rearrangement was created by a simple exchange process. Our computer search indicated that intron 9 and intron 16 contain Alu sequences and that the 44-nucleotide sequence element involved in the rearrangement of the lysyl hydroxylase gene forms a part of the right subunit of the Alu sequence.

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Intron 9     ActgagaCTAcaggCaTgcgcCatCAcaaCtggctaatTTTTtgTaTTTt
Proband      AtatctcCTAtgtaCtTcaagCccCAaccCacctctgaTTTTctTtTTTc
Intron 16    AtatctcCTAtgtaCtTcaagCccCAaccCacctctgaTTTTctTtTTTc

Intron 9     TTTTTTTTTTTTTgagAcGGAGTCTtGCTcTGTtGCCCAGGCTGGAGTGC
Proband      TTTTTTTTTTTTTgaAtGGAGTCTcGCTgTGTcGCCCAGGCTGGAGTGC
Intron 16    TTTTTTTTTTTTTgaAtGGAGTCTcGCTgTGTcGCCCAGGCTGGAGTGC

Intron 9     AaTGGCGtgATCTCAGCTCAgTGCAACCTctGCCTCCCAGGTTCaaGCAA
Proband      AgTGGCGccATCTCAGCTCAcTGCAACCTtcGCCTCCCAGGTTCttGCAA
Intron 16    AgTGGCGccATCTCAGCTCAcTGCAACCTtcGCCTCCCAGGTTCttGCAA

Intron 9     TTCTCcTGCCTCAGCCTCCCGAGTAGCTGGGATTACAGGCGCCTGCCACC
Proband      TTCTCtTGCCTCAGCCTCCCGAGTAGCTGGGATTACAGGCGCCTGCCACC
Intron 16    TTCTCtTGCCTCAGCCTCCCGAGTAGCTGGGATTACAGGCGCCTGCCACC

Intron 9     gTGCCtGgCTAATTTTTtgtatTTTAGTAGAGATaGGgTTtcaCtaTgTt
Proband      gTGCCtGgCTAATTTTTtgtatTTTAGTAGAGATaGGgTTtcaCtaTgTt
Intron 16    aTGCCcGaCTAATTTTTgtataTTTAGTAGAGATgGGtTTcacCatTtTg
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Figure 5 Nucleotide sequence across the duplication junction in the proband's gene for lysyl hydroxylase. The normal sequence of intron 9 (the top line in each of the four sets of three lines) and of intron 16 (the bottom line in each set) and the sequence across the duplication junction (middle line in each set) are aligned in the 5′-to-3′ direction. Capital letters indicate the identities between all these sequences, and the sequences of intron 16 and intron 9 that are adjacent to the duplication junction are underlined.

Simple-Sequence-Repeat Polymorphism as a Test for Possible Uniparental Isodisomy

The human lysyl hydroxylase gene has been mapped to chromosome 1 by Southern blot analysis of human-mouse somatic cell hybrids and by in situ hybridization to 1p36.2–1p36.3 (Hautala et al. 1992). The gene was localized to subband 1p36.31 by using FISH on simultaneously R-banded prometaphase chromosomes (Van Roy et al. 1993). In order to exclude uniparental isodisomy as a cause of the apparent homozygosity at the lysyl hydroxylase gene locus in the proband, three different polymorphic markers on chromosome 1 were used to analyze the number of different chromosome 1 sequences present in the proband's genome; these markers—DIS160, DIS312, and FGR (Patel et al. 1992; Engelstein et al. 1993)—have been mapped to the short arm of chromosome 1, and DIS160 and FGR have been mapped to the subchromosomal region where the lysyl hydroxylase gene is located. All the markers are CA dinucleotide repeats. As shown in figure 6, the proband has a 141- and a 139-bp allele of FGR; GM01790, a patient carrying a similar duplication (Hautala et al. 1993), has 141- and 137-bp alleles, whereas homozygous 141-bp alleles were seen in the control cell line. The proband and GMO1790 were heterozygous for the other two markers on the short arm of chromosome 1 (not shown). This finding is not consistent with uniparental isodisomy for chromosome 1 in these two families.

Use of PCR to Identify the Rearrangement

This study demonstrates that an identical large rearrangement is found in two different families that, at least according to our knowledge, are unrelated (Pinnell et al. 1972; Sussman et al. 1974), suggesting that the mutated allele may be found in the general population. In order to determine whether this duplication is a common rearrangement in the genome, 40 Finnish blood samples and 52 blood samples from U.S. citizens—i.e., 184 alleles from two different countries—were analyzed by PCR using oligonucleotides JH71 and JH55 (see above), which, in the case of this duplication, generate a 300-bp fragment from the junction region of the lysyl hydroxylase gene. Screening of the samples yielded no positive finding.

Analysis of genomic DNA from GM01790 cells, which are from a patient carrying a similar duplication rearrangement in the lysyl hydroxylase gene (Hautala et al. 1993), also resulted in the amplification of the duplication junction fragment when the oligonucleotide primers JH71 and JH55 were used (fig. 4, *bottom*). This result confirms the identity of the rearrangement between these two cell lines. In addition, the results indicate that the PCR assay developed in this study is useful for the detection of the rearrangement in the lysyl hydroxylase gene by direct analysis of genomic DNA (i.e., DNA isolated from a blood sample) of the patient.

We have used our PCR assay to see whether the same duplication of the lysyl hydroxylase gene is also found in the genome of five other EDS type VI patients, the samples of whom were available to us. In order to exclude the type VIB variant of the syndrome, only patients with lysyl hydroxylase activity ≤20% were selected for analysis. None of the five patients studied were positive for the duplication.

Discussion

Our results indicate that in a patient (ATCC CRL 1195) originally analyzed by Sussman et al. (1974), the lysyl hydroxylase gene is rearranged and contains a duplication of the seven exons. The duplication appears to be identical to that which we have described in another EDS type VI family (Hautala et al. 1993). Both cases were originally reported ~20 years ago, and skin fibroblasts of these pa-

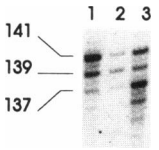

Figure 6 Simple-sequence-repeat polymorphism on chromosome 1. DNA extracted from control cells (lane 1), CRL1195 cells (lane 2), and GM01790 cells (lane 3) was amplified by FGR (Patel et al. 1992) repeat marker. A known sequence ladder was used as a size reference. Multiple bands are seen on the gel, because the *Taq* DNA polymerase may skip individual dinucleotide repeats. The most intense bands are considered to be representatives of the full-length allele (Spotila et al. 1992). Lane 1 shows a 141 homozygote, lane 2 a 141/139 heterozygote, and lane 3 a 141/137 heterozygote.

tients (ATCC CRL 1195 and GM01790) have been stored in two different cell repositories. In order to confirm that these cells are indeed of separate origin, we performed fingerprint analyses of these two cell lines. On the basis of our results, it is evident that the cells originate from different patients. The presence of a similar rearrangement in two unrelated families suggests that the mutated allele may be found in the general population.

EDS type VI has an autosomal recessive form of inheritance. Both cases with the duplication in the gene for lysyl hydroxylase revealed an apparent homozygosity. There was no possibility to study segregation of the mutation in the families, although it seems probable that both parents of each of the patients were heterozygous with respect to the duplication. Uniparental disomy is the inheritance of two homologous chromosomes from the same parent, and this phenomenon has been found to be involved in a gene defect in some chromosome 7 (Spence et al. 1988; Voss et al. 1989; Spotila et al. 1992) and chromosome 15 (Nicholls et al. 1989; Malcolm et al. 1991) mutations. Uniparental disomy frequently results in homozygosity for some segments of the uniparentally derived chromosome pair, a condition called "isodisomy." Our simple-sequence-repeat–polymorphism analysis indicates that the patients with the duplication in the lysyl hydroxylase gene were heterozygous for all three markers on the short arm of chromosome 1. Two of the markers were located at the same subchromosomal region as was the lysyl hydroxylase gene. This finding is not consistent with uniparental isodisomy of the short arm of chromosome 1 and thus strongly suggests that an apparent homozygosity of the duplication of the lysyl hydroxylase gene cannot be explained by uniparental isodisomy. There is no data about polymorphic markers inside the lysyl hydroxylase gene. Unless the genetic distance between the marker and the lysyl hydroxylase gene is very small, the possibility remains, however, that the cosegregating marker allele and the mutation were separated by recombination.

Screening of cells from five additional EDS type VI patients cultured in our laboratory showed no additional cases with the duplication. Although no additional cases were found among these patients, the duplication may be a common cause of the disease. The recessive inheritance of the disorder requires both alleles to be defective. The patients with the duplication are obviously homozygous, the uniparental isodisomy is not probable, and the parents are unrelated. Therefore the duplication rearrangement appears to have occurred at least four times independently. Only one mutation causing the type VIA EDS, a point mutation creating a premature stop codon (Hyland et al. 1992), has been published in addition to the duplication rearrangement. Very recently a patient with both a point mutation in one allele and a 3-nucleotide deletion of another lysyl hydroxylase allele has also been described (Ha et al. 1994). The parents in the family studied by Hyland et al. (1992) and in two other families that were screened in this study are known to be closely related. The data suggest that different kinds of mutations in the lysyl hydroxylase gene may cause the disease, but the duplication rearrangement appears to be more common than the others. Analysis of 40 healthy Finnish individuals and 52 healthy blood donors in the United States gave no positive findings in the screening of the duplication in the general population. Because of the rarity of EDS type VI, this negative result is not surprising.

Major gene rearrangements are not generally a cause of mutation in inherited diseases. However, there are some cases—e.g., Duchenne and Becker muscular dystrophies—where rearrangements are common. In Duchenne muscular dystrophy, partial gene duplications account for 6%–7% of the mutations (Hu et al. 1991), with the duplicated area consisting of 2–22 exons. Duplications were found to result from recombination of either nonhomologous or homologous sequences (Hu et al. 1991). A duplication event caused by a nonhomologous exchange event has also been reported in the gene for lipoprotein lipase (Devlin et al. 1990), and a duplication event caused by a homologous exchange event has been reported both in the gene for the LDL receptor (Lehrman et al. 1987) and in the gene for hypoxanthine phosphoribosyltransferase (Marcus et al. 1993). Alu sequences are involved in the generation of gross rearrangements in many cases (Lehrman et al. 1987; Hu et al. 1991; Marcus et al. 1993), but these sequences are not an absolute requirement for the event. A typical human Alu family member is a sequence ~300 bp long and consists of two similar but not identical subunits designated the "left" and "right" monomers (Jurka and Smith 1988). In the present paper, we have described a homologous recombination in the gene for lysyl hydroxylase in two nonrelated EDS type VI patients. This recombination has occurred via two identical 44-nucleotide-long sequences, one located in intron 9 and the other located in intron 16, in the normal lysyl hydroxylase gene. These identical sequences are parts of the right subunit of the Alu sequences, and both Alu sequences are located in the noncoding strand of the introns. It is interesting to note that the breakpoints of the duplications in both the LDL receptor gene and the dystrophin gene, although located in the left arm of the Alu sequence, are found in the same region of the Alu elements. This region contains promoter sequences of RNA polymerase III and is found to be more susceptible to recombination than are the other parts of the Alu sequence (Hu and Worton 1992).

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