Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 3889–3893, May 1990
Genetics

Tandem duplication within a type II collagen gene (*COL2A1*) exon in an individual with spondyloepiphyseal dysplasia

(fibrillar collagen/chondrodysplasia/DNA sequence analysis/mutation/genetics)

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Communicated by Victor A. McKusick, February 23, 1990

ABSTRACT **We have characterized a mutation in the type II collagen gene (*COL2A1*) that produces a form of spondyloepiphyseal dysplasia. The mutation is an internal tandem duplication of 45 base pairs within exon 48 and results in the addition of 15 amino acids to the triple-helical domain of the α1 chains of type II collagen derived from the abnormal allele. Although the repeating (Gly-Xaa-Yaa)$_n$ motif that characterizes the triple-helical domain is preserved, type II collagen derived from cartilage of the affected individual contains a population with excessive posttranslational modification, consistent with a disruption in triple-helix structure. The mutation is not carried by either parent, indicating that the phenotype in the affected individual is due to a new dominant mutation. DNA sequence homology in the area of the duplication suggests that the mutation may have arisen by unequal crossover between related sequences, a proposed mechanism in the evolution and diversification of the collagen gene family.**

The spondyloepiphyseal dysplasias (SEDs) are a heterogeneous subgroup of the skeletal dysplasias whose cardinal features include abnormal epiphyses, flattened vertebral bodies, and ocular involvement that ranges from myopia to vitreo-retinal degeneration (1). Since type II collagen is found in a restricted set of tissues that includes articular cartilage, the nucleus pulposus of the spine, and the vitreous of the eye (2), the concordance between the clinical findings and the distribution of the protein suggests that a primary defect of type II collagen may be responsible for some of these disorders. Structurally abnormal type II collagen has been isolated from cartilage of individuals with SED (3, 4), spondyloepimetaphyseal dysplasia (3, 4), and achondrogenesis–hypochondrogenesis (5). Combined with the demonstration of linkage of markers in the *COL2A1* gene with a form of familial osteoarthritis (6) and with Stickler syndrome (7, 8), these studies have suggested that mutations in the type II collagen gene may underlie a spectrum of disorders that span a broad range of clinical severity.

Type II collagen is a fibrillar collagen that in its mature form is a homotrimer of α1(II) chains (9). Biochemical studies have shown that cartilage from individuals with either SED (3, 4) or achondrogenesis–hypochondrogenesis (5) contains type II collagen with both slowly migrating and normally migrating α1(II) chains. Amino acid analyses of the abnormal type II collagen from the affected individuals have shown that there is increased lysyl hydroxylation (3–5), suggesting that the more slowly migrating population is derived from molecules containing a chain with a defect that affects triple-helix structure and/or assembly. These results are conceptually homologous to the consequences of defects in type I collagen that produce osteogenesis imperfecta, in which mutations that alter triple-helix structure result in excessive posttranslational modification of all chains in molecules that incorporate at least one abnormal chain (10, 11). The type I collagen studies have also shown a close correlation between the location of mutation and the carboxyl-terminal limit of overmodification (12–15), consistent with the carboxyl- to amino-terminal polarity of triple-helix assembly.

Two mutations in the *COL2A1* gene have been reported in individuals with chondrodysplasias. Affected members of one family with dominantly inherited SED have been shown to carry an intron-to-intron deletion spanning exon 48, resulting in deletion of 36 amino acids from the carboxyl-terminal end of the triple helix (16). An infant with the lethal achondrogenesis–hypochondrogenesis phenotype was shown to carry a point mutation that resulted in substitution of serine for glycine at residue 943 of the triple helix (17).

Here we have used analysis of peptides resulting from CNBr cleavage of type II collagen (CNBr peptides) from a patient with SED to localize overmodification to a specific region of the protein and analyzed the corresponding region in genomic DNA. We show that the patient is heterozygous for a 45-base-pair (bp) tandem duplication within a *COL2A1* exon. The sequence of the mutation implies a protein with a triple-helical domain 15 amino acids longer than normal but with preservation of the canonical Gly-Xaa-Yaa repeating motif. The mutation arose in the maternally derived allele and, based on substantial DNA sequence homology in the region of the duplication, may have arisen by unequal crossover between misaligned *COL2A1* alleles.

MATERIALS AND METHODS

Clinical Summary. The proband is the son of clinically normal parents and has an unaffected sister. Diagnosis was made at 6 years of age on the basis of disproportionate short stature (height of 92 cm, four standard deviations below the mean; upper/lower segment ratio of 1.2), scoliosis, myopia, and pectus excavatum. Radiographic examination showed small, irregular epiphyses and platyspondyly. The iliac crest biopsy from which the type II collagen was isolated was performed when the patient was 7 years old.

Collagen Extraction and Protein Analysis. Collagen was extracted as described (4). Cartilage from a normal fetal control and the SED patient was minced and extracted for 24 hr with 4 M guanidine hydrochloride containing 1 mM phenylmethylsulfonyl fluoride, 10 mM *N*-ethylmaleimide, and 25 mM EDTA as protease inhibitors. Tissue was washed with water, and the type II collagen was extracted with pepsin in 0.5 M acetic acid/0.2 M NaCl based on a pepsin/

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Abbreviations: SED, spondyloepiphyseal dysplasia; PCR, polymerase chain reaction.

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tissue ratio of 5:1000 (5 μg of pepsin per mg of tissue). After 48 hr at 4°C, the digest was centrifuged to remove debris. The supernatant contained the type II collagen.

Intact chains were analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) in 5% gels (18) and were visualized by silver staining (19). Cleavage with CNBr was as described (20), with the CNBr peptides analyzed by SDS/PAGE in 10% gels and visualized by silver staining. To retain the lower molecular weight peptides in the gels, 10% (vol/vol) methanol was included in all water washes (4).

Amplification of Genomic DNA. Polymerase chain reaction (PCR) amplifications (21) contained 500 ng of genomic DNA in 100 μl of 50 mM KCl/10 mM Tris, pH 8.3/5 mM $MgCl_2$/0.01% gelatin/400 μM each dNTP/0.5 μM each oligonucleotide primer/5 units of *Thermus aquaticus* (*Taq*) polymerase (Amplitaq, Perkin–Elmer/Cetus). After an initial incubation at 94°C for 2 min, 40 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min 45 sec at 72°C were performed in a Perkin–Elmer DNA thermal cycler. Products were separated by gel electrophoresis through 6% polyacrylamide gels, stained with ethidium bromide, and photographed.

Cloning and DNA Sequence Analysis. Preparative amounts of amplified DNA were separated by electrophoresis through a 2% low-melting-point agarose (BRL) gel, and the amplified product was resected and purified (22). The fragment was cleaved with *Bcl* I and ligated with *Bam*HI/*Hin*cII-cleaved M13 mp18. DNA sequence was determined by the chain-termination method (23) using Sequenase (United States Biochemical). DNA sequences were analyzed by using GCG (24) and software purchased from IBI.

DNA Hybridization. Genomic DNA was cleaved with restriction enzymes according to the manufacturer's specifications, separated by agarose gel electrophoresis, and transferred to Nytran (Schleicher & Schuell) by vacuum pressure with a Vacugene (LKB-Pharmacia) apparatus (25). Filters were hybridized with *COL2A1* genomic DNA fragments (26) labeled with [α-^{32}P]dCTP (DuPont/NEN) by random primer extension (27).

RESULTS

Structurally Abnormal Type II Collagen. Electrophoretic analysis showed that α chains of type II collagen derived from cartilage of the SED patient migrated as a doublet composed of a population of normal mobility and a second population of slower mobility (Fig. 1*A*). The relative amount of normally migrating material was greater than that seen in previously reported cases (3, 4). The purified type II collagen molecules were cleaved with CNBr, and the resulting peptides were separated by gel electrophoresis (Fig. 1*B*). For each of the CNBr peptides, the band was abnormally broad, suggesting a doublet composed of normally migrating and more slowly migrating fragments. These were presumably derived from the corresponding normally and slowly migrating α chains, respectively. The band corresponding to peptide 9, 7 was particularly broad. These data suggested that the slowly migrating population of α chains was of higher molecular weight because of an increase in posttranslational prolyl and lysyl hydroxylation and hydroxylysyl glycosylation (overmodification) within the triple-helical domain. In addition, because all of the peptides that resulted from CNBr cleavage contained an apparently overmodified component, the results imply that there is a defect near the carboxyl-terminal portion of the triple-helical domain that interferes with the formation and/or structure of the triple helix.

An Insertion in the *COL2A1* Gene. The carboxyl-terminal portion of the triple-helical domain, including CNBr peptide 9, 7 and most of the carboxyl-terminal propeptide, is encoded by exons 45–51, a region spanning approximately 3.3 kilobases (kb) (28). By PCR (21), we amplified this region of the *COL2A1* gene and examined the products of the reactions by PAGE. For one of the amplified regions, containing sequences from exons 48 and 49, DNA from the patient yielded two fragments, one that comigrated with fragments amplified from control DNA and an additional fragment about 30–50 bp longer (Fig. 2). Amplification with primers internal to those indicated in Fig. 2 and analysis of restriction enzyme digests of the amplified material localized the insertion to exon 48 (data not shown). The larger amplified fragment is not seen in amplified DNA from either parent, implying that the SED phenotype in the patient was the result of a new dominant mutation.

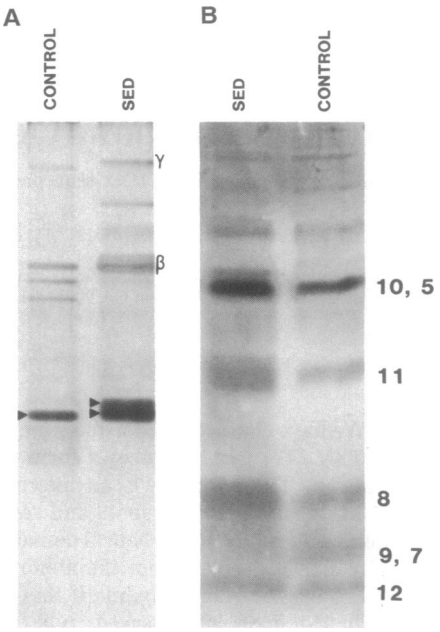


FIG. 1. Electrophoretic analysis of type II collagen. (*A*) α chains separated by SDS/PAGE (arrowheads). The positions of the β and γ chains are also indicated. (*B*) CNBr peptide fragments, identified by number.

In addition to the normal and insertion-carrying fragments, a pair of fragments of much higher apparent molecular weight were observed in amplified DNA from the patient. Amplification of the individual alleles (see Fig. 4*B*) did not yield these fragments, suggesting that their formation is dependent on the presence of both alleles and that they represent interallelic heteroduplexes between complementary strands of the amplified products of the two alleles. We and others (29) have also observed such anomalously migrating heteroduplexes in amplified DNA fragments from individuals heterozygous for deletions as small as 5 bp (30) and as large as 108 bp (D.H.C., unpublished data).

Internal Duplication Within a *COL2A1* Exon. A fragment of the amplified DNA was subcloned into a bacteriophage M13 vector, and the nucleotide sequences of the normal allele and the allele with the insertion were determined (Fig. 3). The DNA sequence revealed that the insertion was due to an internal tandem duplication of 45 bp within exon 48. The implied protein product of this allele contains an additional 15 amino acids within the triple-helical domain and maintains the repeating Gly-Xaa-Yaa pattern essential to triple-helix formation.

Origin of the Mutation. To determine the parental origin of the allele carrying the mutation, we analyzed restriction fragment length polymorphisms within the *COL2A1* gene. The affected individual was heterozygous (+/−) for a *Hin*dIII polymorphic site, his mother was a (+/+) homozygote, and his father was a (−/−) homozygote (Fig. 4*A*). Since the *Hin*dIII fragments recognized by the probe also carried the region of the gene encoding exon 48, we used agarose gel

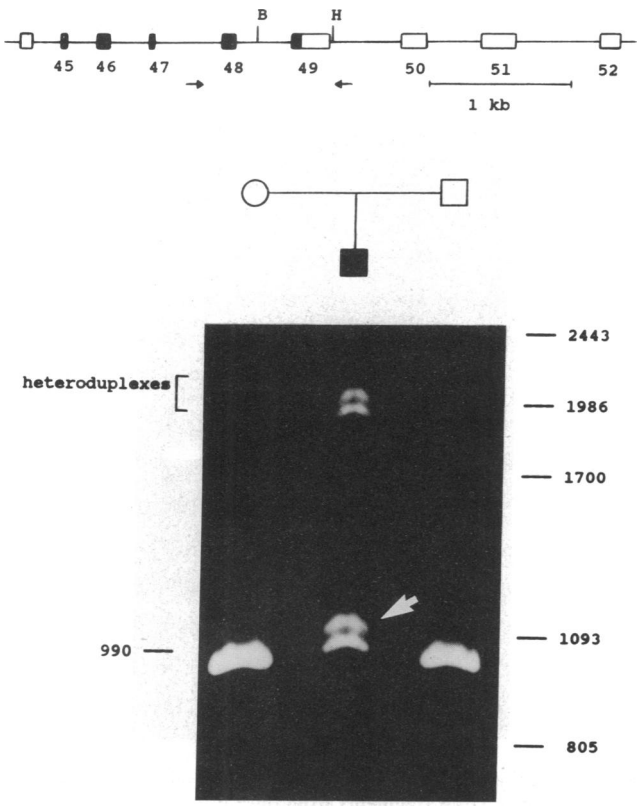
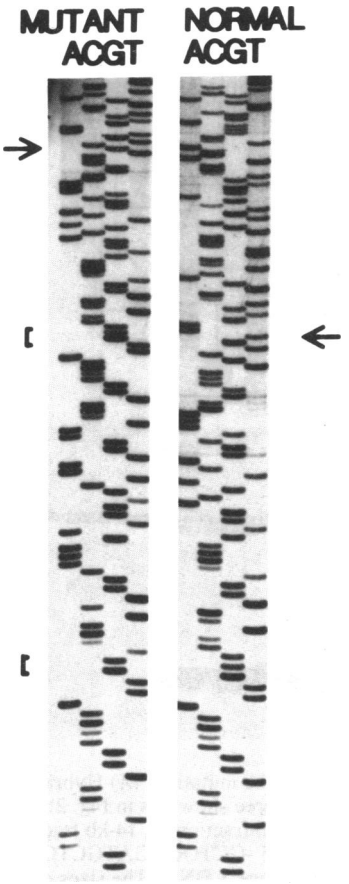


FIG. 2. PCR amplification. (*Upper*) Structure of the 3′ end of the *COL2A1* gene. Boxes represent exons, which are identified by number. Solid areas within the boxes indicate the exonic regions of *COL2A1* that encode CNBr fragment 9, 7 of the α1 chain of type II collagen. The arrows represent the oligonucleotide primers used for amplifying the 990-bp fragment from the patient and his parents. B marks a unique *Bcl* I site within the amplified fragment used in subcloning. H marks the 3′ nonpolymorphic *Hin*dIII site used in the analysis of *COL2A1* restriction fragment length polymorphisms (see Fig. 4). The oligonucleotide primer sequences are: 5′ primer, 5′-TTCCATGACTGAGCATGTGAAGAAC-3′; 3′ primer, 5′-CAGGCCCAGCTCTGCCCTGTACTAG-3′. (*Lower*) Amplified fragments separated by PAGE. The pedigree is shown above the lanes, with the filled square indicating the affected individual. The additional fragment present in the proband (white arrow) and the interallelic heteroduplexes (bracket) are identified. Molecular weight markers are from a *Pst* I digest of bacteriophage λ DNA.

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    GGT CCT CCT GGC CCC GTC [GGT CCC TCT GGC AAA GAT GGT
964 Gly Pro Pro Gly Pro Val [Gly Pro Ser Gly Lys Asp Gly

    GCT AAT GGA ATC CCT GGC CCC ATT] [GGT CCC TCT GGC AAA
    Ala Asn Gly Ile Pro Gly Pro Ile] [Gly Pro Ser Gly Lys

    GAT GGT GCT AAT GGA ATC CCT GGC CCC ATT] GGG CCT CCT
    Asp Gly Ala Asn Gly Ile Pro Gly Pro Ile] Gly Pro Pro

    GGT CCC CGT GGA CGA TCA GGC GAA ACC GGC CCT GCT
    Gly Pro Arg Gly Arg Ser Gly Glu Thr Gly Pro Ala
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FIG. 3. DNA sequence analysis. The portion of the abnormal allele containing the duplication is shown with its normal counterpart. The sequence begins with the codon for the glycine residue at position 979 of the triple-helical domain (the first glycine of the triple helix is defined by convention as residue 1). The duplication junctions in the abnormal allele are bracketed. Arrows mark the 3′ ends of exon 48 in the normal and abnormal alleles. The entire sequence of exon 48 from the abnormal allele and the implied protein sequence are shown below with the duplicated units underlined and bracketed. Exon 48 encodes residues 964–999 of the triple-helical domain. Residues 970–984 are duplicated.

electrophoresis to separate and purify fractions containing the 7-kb (+ allele) and 14-kb (− allele) fragments from *Hin*dIII-digested genomic DNA from the patient. Amplification of the two fractions by PCR showed that the insertion was contained within the 7-kb (+ allele) fraction, demonstrating that the maternally derived allele carried the insertion (Fig. 4*B*). The mother was homozygous for polymorphic markers to the 5′ side of exon 48, so we were unable to determine if the mutation arose by recombination between alleles at meiosis.

DISCUSSION

We have shown that an individual with SED carries a duplication of a 45-bp segment within an exon that encodes a portion of the triple-helical domain of one *COL2A1* allele. The mutation resulted in synthesis of a population of type II collagen molecules that are posttranslationally overmodified throughout the triple-helical domain. We have used this observation to localize the mutation to the region of the gene that encodes the most carboxyl-terminal cyanogen bromide peptide fragment of the triple-helical domain.

From our studies, we cannot determine whether the overmodified molecules are derived from homotrimers of the longer α chains, trimers containing both normal and longer α chains, or both. We expect that trimers containing both normal and longer chains will be overmodified, poorly secreted, and perhaps degraded intracellularly. Reduced secretion has been observed in collagens synthesized by cells from individuals heterozygous for large deletions within the triple-helical domains of both type I and type III collagen genes (10, 31–34). Inefficient secretion of overmodified chains is further supported by the observation that chondrocytes from some individuals with SED have markedly distended rough endoplasmic reticulum (35).

We have shown that the mutation arose in the maternally derived *COL2A1* allele. The structure of the mutation is consistent with the occurrence of an unequal crossover

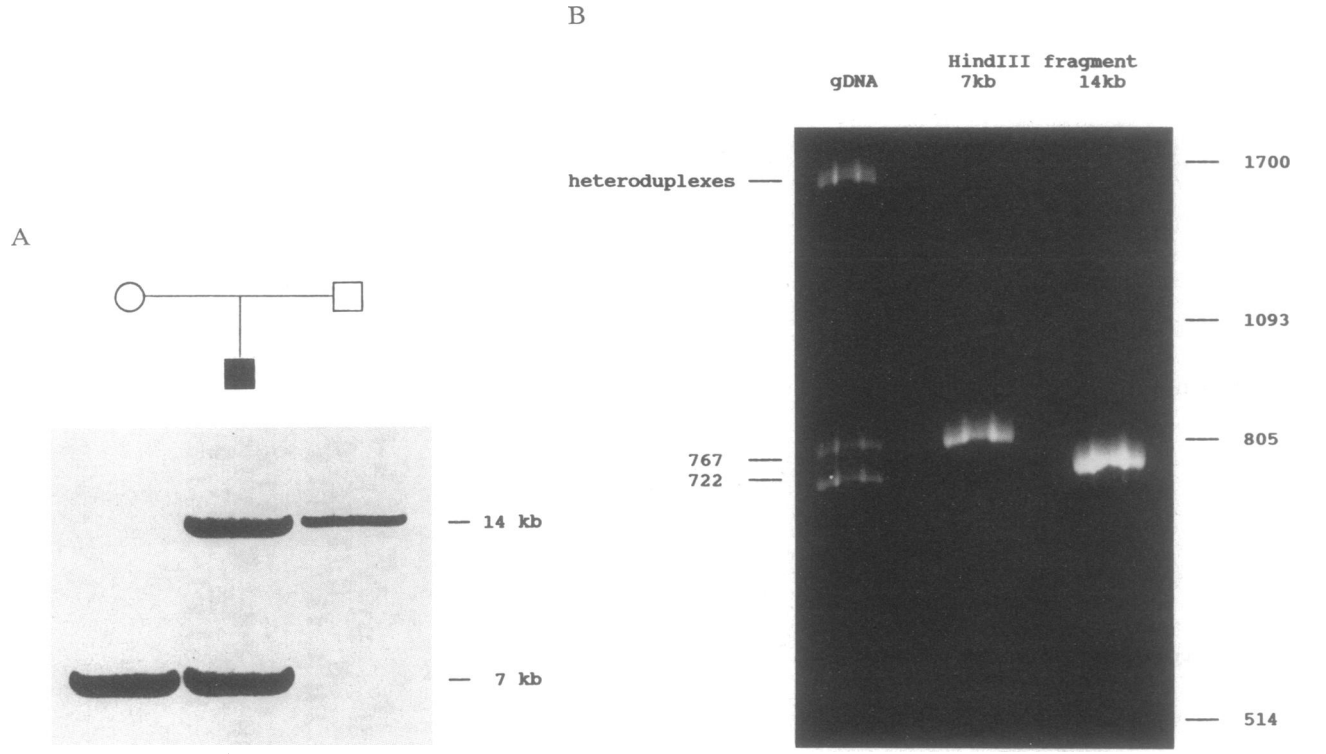

FIG. 4. Origin of the mutation. (*A*) Hybridization of a *COL2A1* genomic DNA probe to a *Hin*dIII digest of genomic DNA from the patient and his parents (pedigree shown as in Fig. 2). (*B*) PCR amplification of *Hin*dIII genomic DNA fragments from the SED patient. Lanes 1, total genomic DNA; 2, 7-kb fraction; 3, 14-kb fraction. The oligonucleotide primer sequences are: 5′ primer, 5′-CTGTGGTGACCACTCTTTCCTCACG-3′; 3′ primer, 5′-GCTGCGGATGCTCTCAATCTGGTTG-3′. Molecular weight markers on the right margin of the figure are from a *Pst* I digest of bacteriophage λ DNA. The sizes of amplified DNA fragments from the normal and insertion-carrying allele are indicated as are the heteroduplexes in the lane containing amplified genomic DNA.

between *COL2A1* sequences within exon 48. Alignment of two copies of exon 48 (Fig. 5) in the manner that would have preceded the recombinational event shows 18 of 23 homologous bases (78%) surrounding the point of recombination. Excluding the first two nucleotides of the glycine codons there are still 12 of 17 (71%) homologous bases. The homology is most striking to the 5′ side of the putative region of exchange.

Most of the triple helical domains of the fibrillar collagens are encoded by 42 exons, many of which are 54 bp long or a multiple of 54 bp in length (36, 37). It has been hypothesized that this structure arose by tandem duplication of an ancestral 54-bp exon (36). The internally repetitive nature of fibrillar collagen gene exons has suggested that recombination between Gly-Xaa-Yaa-encoding sequences may have been responsible for the generation of the 54-bp ancestral exon from a 9-bp precursor (38) as well as exon sizes different from the 54-base-pair unit (36). Surprisingly, the deletions described to date in the type I and type III collagen genes have their endpoints within introns (10, 31, 32). Our data provide evidence that recombination between related exon sequences can occur but also show that the consequences of the event in an individual heterozygous for the resulting mutation may be deleterious.

In addition to this duplication in exon 48, a mutation that results in deletion of the same exon in a family with dominantly inherited SED has also been described (16). In other individuals with SED in which there is a population of type II collagen overmodified over the entire triple helix, we have found neither insertions nor deletions in the region of the gene encoding CNBr fragment 9, 7 (G.E.T. and D.H.C., unpublished data). In addition, similarly overmodified type II collagen has been isolated from infants with the lethal achondrogenesis–hypochondrogenesis phenotype (5, 39), and one such infant has been shown to be heterozygous for a point mutation that changes the glycine at residue 943 of the triple-helical domain to serine (17). These data support the genetic and biochemical studies indicating that heterozygosity for dominant mutations in *COL2A1* is responsible for this group of disorders but suggest that it is too early to relate mutation to phenotype. The approach we have described will be useful in defining additional mutations that produce a similar biochemical phenotype. The characterization of mutations from individuals with biochemically similar but clinically disparate phenotypes may provide a molecular ratio-

FIG. 5. Proposed mechanism for the generation of the abnormal allele. Two copies of exon 48 are aligned to compare the homology surrounding the presumed region of exchange. Uppercase letters are used for the exon, while lowercase letters are sequences from the flanking introns. Homologous bases are indicated by a vertical line. The regions of each allele present in the presumed recombinational product are boldface and underlined. There is overlap between the underlined portions of the two alleles to indicate that the precise point of exchange cannot be determined from the sequence. The X marks the region of exchange. The bottom line shows the sequence of the allele that would result from the recombinational event.

nale that explains the clinical differences and suggests functional roles for regions of the type II collagen molecule in the extracellular matrix.

We thank the patient and his family for their interest and participation. We also thank Jose Bautista, Steve Hwang, John Carrington, and Jeff Greenwald for expert technical assistance. We are indebted to D. Prockop for sharing *COL2A1* sequence information prior to its publication. We thank B. Lee and F. Ramirez for providing *COL2A1* probes and for useful and stimulating discussions. This work was supported by a grant from the National Institutes of Health (HD22657) and by a Biomedical Research Support Grant (S07RR05468). G.E.T. was supported by a National Institutes of Health Training Grant (GM 08243) to the University of California, Los Angeles, Intercampus Training Program in Medical Genetics.

1. Rimoin, D. L. & Lachman, R. S. (1983) in *Principles and Practice of Medical Genetics*, eds. Emery, A. E. H. & Rimoin, D. L. (Churchill Livingston, New York), pp. 703–735.
2. Miller, E. J. & Gay, S. (1987) *Methods Enzymol.* **144,** 3–41.
3. Murray, L. W. & Rimoin, D. L. (1988) *Pathol. Immunopathol. Res.* **7,** 99–103.
4. Murray, L. W., Bautista, J., James, P. L. & Rimoin, D. L. (1989) *Am. J. Hum. Genet.* **45,** 5–15.
5. Godfrey, M. & Hollister, D. W. (1988) *Am. J. Hum. Genet.* **43,** 904–913.
6. Palotie, A., Ott, J., Elima, K., Cheah, K., Vaisanen, P., Ryhanen, L., Vikkula, M., Vuorio, E. & Peltonen, L. (1989) *Lancet* **i,** 924–927.
7. Francomano, C. A., Lieberfarb, R. M., Hirose, T., Maumenee, I., Streeten, E. A., Meyers, D. A. & Pyeritz, R. E. (1987) *Genomics* **1,** 293–296.
8. Knowlton, R. G., Weaver, E. J., Struyk, A. F., Knobloch, W. H., King, R. A., Norris, K., Shamban, A., Uitto, J., Jimenez, S. A. & Prockop, D. J. (1989) *Am. J. Hum. Genet.* **45,** 681–688.
9. Bornstein, P. & Sage, H. (1980) *Annu. Rev. Biochem.* **49,** 957–1003.
10. Willing, M. C., Cohn, D. H., Starman, B., Holbrook, K. A., Greenberg, C. R. & Byers, P. H. (1988) *J. Biol. Chem.* **263,** 8398–8404.
11. Byers, P. H. (1989) in *The Metabolic Basis of Inherited Disease*, eds. Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D. (McGraw-Hill, New York), 6th Ed., pp. 2805–2842.
12. Bonadio, J. F. & Byers, P. H. (1985) *Nature (London)* **316,** 363–366.
13. Cohn, D. H., Byers, P. H., Steinmann, B. & Gelinas, R. E. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6045–6047.
14. Bateman, J. F., Chan, D., Walker, I. D., Rogers, J. G. & Cole, W. G. (1987) *J. Biol. Chem.* **262,** 7021–7027.
15. Vogel, B. E., Minor, R. R., Freund, M. & Prockop, D. J. (1987) *J. Biol. Chem.* **262,** 14737–14744.
16. Lee, B., Vissing, H., Ramirez, F., Rogers, D. & Rimoin, D. L. (1989) *Science* **244,** 978–980.
17. Vissing, H., D'Alessio, M., Lee, B., Ramirez, F., Godfrey, M. & Hollister, D. W. (1989) *J. Biol. Chem.* **264,** 18265–18267.
18. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
19. Oakley, B. R., Kirsch, D. R. & Morris, N. R. (1980) *Anal. Biochem.* **110,** 201–207.
20. Bentz, H., Morris, N. R., Murray, L. W., Sakai, L. Y., Hollister, D. W. & Burgeson, R. E. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3168–3172.
21. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1987) *Science* **239,** 487–491.
22. Benson, S. A. (1984) *BioTechniques* **2,** 66–67.
23. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
24. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–395.
25. Southern, E. (1975) *J. Mol. Biol.* **98,** 503–517.
26. Sangiorgi, F. O., Benson-Chanda, V., deWet, W. J., Sobel, M. E., Tsipouras, P. & Ramirez, F. (1985) *Nucleic Acids Res.* **13,** 2207–2225.
27. Feinberg, A. & Vogelstein, B. (1984) *Anal. Biochem.* **137,** 266–267.
28. Cheah, K. S. E., Stoker, N. G., Griffin, J. R., Grosveld, F. G. & Solomon, E. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2555–2559.
29. Nagamine, C. M., Chan, K. & Lau, Y.-F. C. (1989) *Am. J. Hum. Genet.* **45,** 337–339.
30. Willing, M. C., Cohn, D. H. & Byers, P. H. (1990) *J. Clin. Invest.* **85,** 282–290.
31. Barsh, G. S., Roush, C., Bonadio, J. F., Byers, P. H. & Gelinas, R. E. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2870–2874.
32. Chu, M.-L., Gargiulo, V., Williams, C. J. & Ramirez, F. (1985) *J. Biol. Chem.* **260,** 691–694.
33. Superti-Furga, A., Gugler, E., Gitzelman, R. & Steinmann, B. (1988) *J. Biol. Chem.* **263,** 6226–6232.
34. Superti-Furga, A., Steinmann, B., Ramirez, F. & Byers, P. H. (1989) *Hum. Genet.* **82,** 104–108.
35. Borochowitz, Z., Ornoy, A., Lachman, R. & Rimoin, D. L. (1986) *Am. J. Med. Genet.* **24,** 273–288.
36. Yamada, Y., Avvedimento, V. E., Mudryj, M., Ohkubo, H., Vogeli, G., Irani, M., Pastan, I. & deCrombrugghe, B. (1980) *Cell* **22,** 887–892.
37. Ramirez, F., Bernard, M., Chu, M.-L., Dickson, L., Sangiorgi, F., Weil, D., deWet, W., Junien, C. & Sobel, M. (1985) *Ann. N.Y. Acad. Sci.* **460,** 117–129.
38. Benveniste-Schrode, K., Doering, J. L., Hauck, W. W., Schrode, J., Kendra, K. L. & Drexler, B. K. (1985) *J. Mol. Evol.* **22,** 209–219.
39. Murray, L. W., James, P. L., Bautista, J. & Rimoin, D. L. (1988) *FASEB J.* **2,** A1589.