A Mutation in COL9A2 Causes Multiple Epiphyseal Dysplasia (EDM2)

YASUTERU MURAGAKI,[a] EDWIN C. M. MARIMAN,[b]
SYLVIA E. C. VAN BEERSUM,[b] MERJA PERÄLÄ,[c]
JAN B. A. VAN MOURIK,[d] MATTHEW L. WARMAN,[e]
BEN C. J. HAMEL,[b] AND BJORN R. OLSEN[a]

[a]*Department of Cell Biology*
Harvard Medical School
Boston, Massachusetts 02115

[b]*Department of Human Genetics*
University Hospital Nijmegen
6500 HB Nijmegen
The Netherlands

[c]*Department of Medical Biochemistry*
University of Turku
20520 Turku
Finland

[d]*Department of Orthopaedic Surgery*
St. Joseph Hospital
5500 MB Veldhoven
The Netherlands

[e]*Department of Genetics*
Case Western Reserve University School of Medicine
Cleveland, Ohio 441067

Multiple epiphyseal dysplasia (MED) is an autosomal dominant skeletal disorder characterized by early onset osteoarthritis with short stature and stubby fingers. Linkage analyses have identified at least three genetic loci for MED: EDM1 on chromosome 19,[1] EDM2 on chromosome 1,[2] and an additional locus.[3] EDM1 is caused by mutations in the cartilage oligomeric matrix protein gene (COMP);[4] mutations in COMP have also been demonstrated in pseudoachondroplasia (PSACH).[5] We examined a large Dutch family with MED and found that the disorders in this family is caused by a mutation in the α2(IX) collagen gene.[6] The characteristic clinical features of affected individuals include pain in the knee joints and waddling gait between 3 and 6 years of age. X rays of knee joints showed the typical changes of multiple epiphyseal dysplasia: flattened, irregular epiphyses, varus/valgus deformity of the knees, and gradually appearing osteoarthritis with or without loose bodies. Linkage analysis with microsatellite markers from the EDM1 (D19S199, D19S212, D19S215, D19S222) and EDM2 (D1S186 and MYCL) regions showed significant linkage between the disease and EDM2.

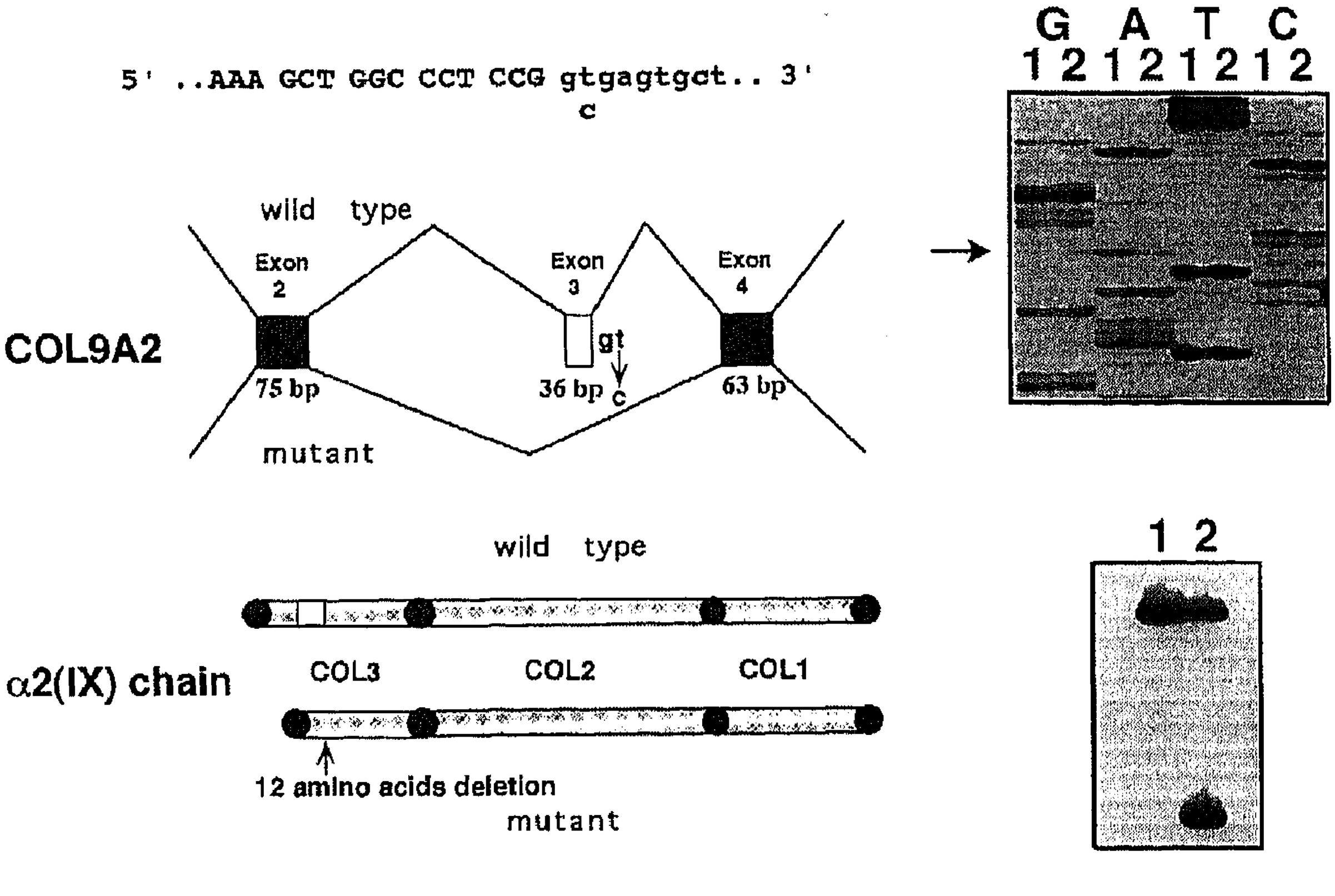

Figure 1. Diagrams showing mutation in splice donor site and exon skipping leading to the in-frame deletion in α2(IX) collagen in EDM2. Above the diagram showing the splice patterns of exons 2, 3, and 4 in wild-type and mutant COL9A2, the nucleotide sequence of the 3′ region of exon 3 (*uppercase letters*) and the 5′ end of intron 3 (*lowercase letters*) is shown. The splice donor sequence *gt* is *gc* in the mutant. The result of cycle sequencing of genomic DNA (antisense strand) from an unaffected individual (lanes 1) and an affected individual (lanes 2) is shown in the upper, right-hand inset. The *arrow* indicates the step in the sequence ladder at which the affected individual is heterozygous for A and G. The result of RT-PCR amplification and acrylamide gel electrophoresis with RNA from an unaffected individual (lane 1) and an affected individual (lane 2) is shown in the lower, right-hand inset. The bottom diagram shows the location of the 12 amino acid residue deletion in the COL3 domain of the α2(IX) collagen chain caused by the mutation.

To find the causative mutation we performed RT-PCR with total RNA from short-term cultured chondrocytes, obtained during arthroscopic surgery, and from EBV transformed lymphoblasts from an affected patient. First-strand cDNAs were synthesized with oligo(dT) primers using the Superscript Preamplification System (GIBCO BRL). Since the EDM2 locus includes COL9A2, PCR primers were designed to amplify the approximately 2-kb cDNA coding for α2(IX) collagen in four overlapping fragments. Second-round PCR had to be used to amplify overlapping cDNA fragments encoding the NC2, COL2, NC3, and COL3 domains and the carboxyl half of the signal peptide of the α2(IX) collagen chain with nested primers. When the PCR products were analyzed on 2% agarose gels all products were the same size from affected and unaffected individuals except one containing coding sequences for the COL3 domain. This product migrated as a single band from unaffected individuals and as a double band from affected individuals. Dideoxy-nucleotide cycle sequenc-

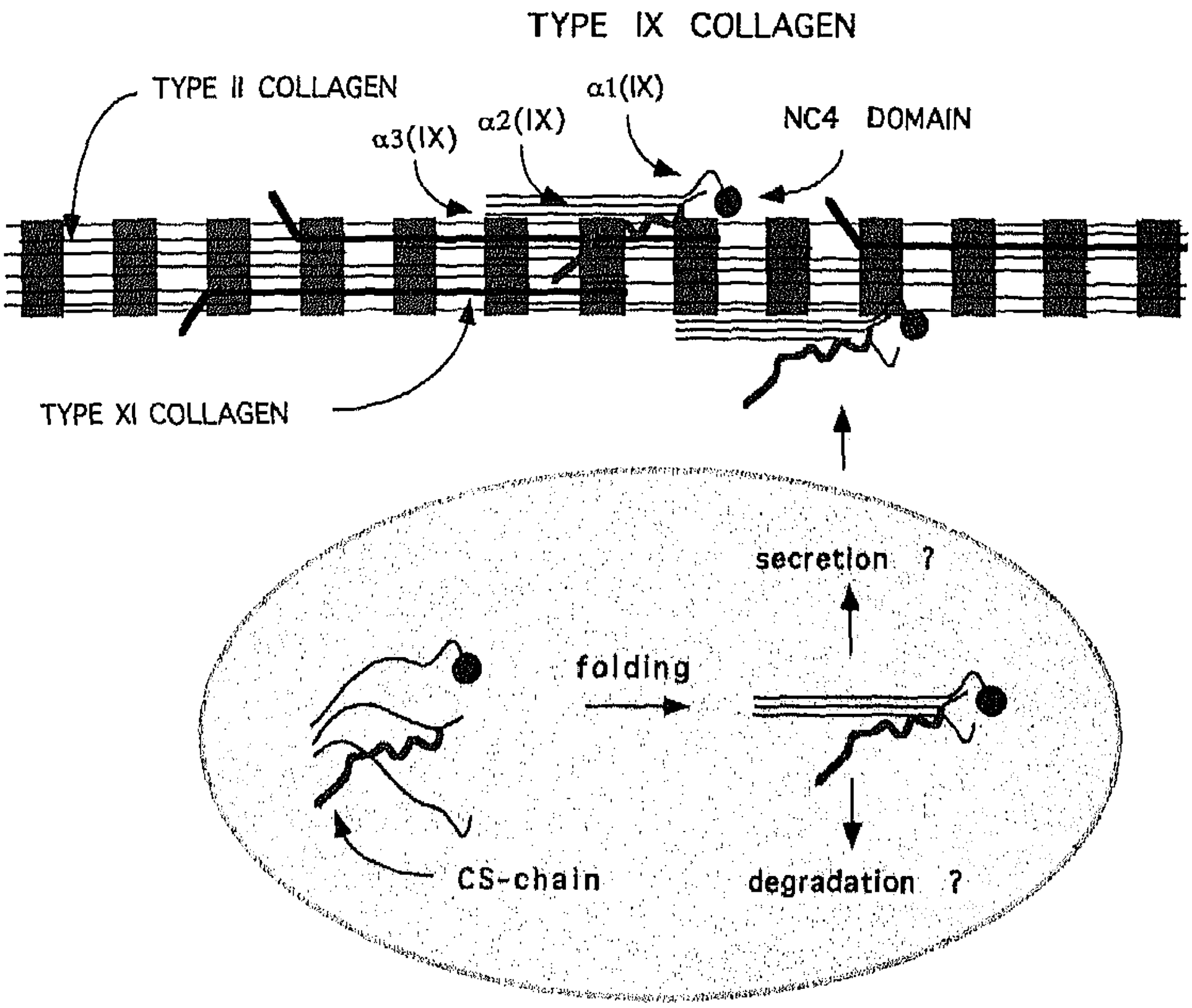

Figure 2. Diagram showing the potential fates of collagen IX trimers containing a mutated α2(IX) chain. Within chondrocytes (*grey oval*) mutated α2(IX) chains are likely to be incorporated into trimers. Such trimers would have a folding defect in the COL3 domain and may be degraded intracellularly, or be secreted and incorporated into fibrils. The abnormal conformation of their COL3 domains may disrupt the normal function of such molecules on the fibrillar surface.

ing (ampliCycle™, Perkin Elmer) showed a 36-nt deletion in the lower band. To better analyze this deletion, second-round PCR was performed with more closely spaced primers; 0.5 μL of [α-^{33}P] dCTP (10 mM, 2000 Ci/m mol) were added to the reaction (FIG. 1). Since the 36 nucleotide deletion corresponded to a single exon encoding part of the amino terminal COL3 domain of the α2(IX) collagen polypeptide (FIG. 1), genomic DNA was PCR amplified for further analysis. Cycle sequencing of the genomic PCR products revealed that the sequence of the splice donor site of intron 3, GTGAG, was converted to GCGAG in one allele of the affected patient (FIG. 1). This nucleotide change, A T-to-C transition, resulted in loss of an HphI site in the genomic sequence of the affected allele. To test for cosegregation of this change with the disorder, we examined the loss of this restriction site in all family members by digesting genomic PCR products with HphI. All affected individuals were heterozygous for this change, while all unaffected individuals were homozygous for the pres-

ence of the HphI site (data not shown). We conclude, therefore, that the MED in this family is caused by a splice donor site mutation in intron 3 of the α2(IX) collagen gene, leading to exon skipping during RNA splicing and an in-frame loss of 12 amino acid residues within a triple-helical domain of the polypeptide. The deletion may not have an effect on polypeptide synthesis or formation of collagen IX trimers; however, the mutation may have a dominant negative effect either at the level of protein secretion or supramolecular assembly (FIG. 2).

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