A mutation in the gene encoding the \( \alpha_2 \) chain of the fibril-associated collagen IX, COL9A2, causes multiple epiphyseal dysplasia (EDM2)


Multiple epiphyseal dysplasia, an autosomal dominant disease, is among the more common inherited osteochondrodysplasias. Symptoms range from stiffness and pain in large joints to frank osteoarthritis associated with short stature and stubby fingers. Linkage analyses of multiple epiphyseal dysplasia families suggest at least three loci. One locus, EDM1, maps to chromosome 19 (ref. 4), and is caused by mutations in cartilage oligomeric matrix protein (COMP). Mutations in COMP have also been identified in patients with pseudoachondroplasia (PSACH), consistent with previous analyses which suggested that EDM1 and PSACH could be allelic disorders. A second locus, EDM2, maps to chromosome 1 in the vicinity of the COL9A2 gene. Finally, exclusion of EDM1 and EDM2 in other families suggests the existence of at least one additional locus. We now show that affected members of a large kindred with multiple epiphyseal dysplasia linked to the EDM2 locus are heterozygous for a splice site mutation within COL9A2, causing exon skipping during RNA splicing and an in-frame loss of 12 amino acid residues within the \( \alpha_2 \) collagen chain. The results provide the first in vivo evidence for the role of collagen IX in human articular cartilage.

Clinical features in part of the present family have been previously reported. Affected individuals typically presented during childhood and adolescence with waddling gait and stiffness and/or pain in the knees. Few patients experienced involvement of other joints such as the elbow, wrist, or ankle. No one complained of hip or shoulder pain. Some patients were mildly short statured and/or had stubby hands. Several patients never sought medical advice because of the mildness of their complaints. There were no spine abnormalities. X-rays revealed flattened, irregular epiphyses (Fig. 1), varus/valgus deformity of the knees, and gradually appearing osteoarthritic with or without loose bodies. Since family members did not have complaints about their hips, X-rays of hips are limited to only a few cases. In these cases (including the patient whose knees are shown in Fig. 1) a variable phenotype is seen, ranging from almost normal for age to slight irregularity of epiphyseal surface and acetabulum.

Linkage analysis was performed with microsatellite markers from the EDM1 (D19S199, D19S212, D19S215, D19S222) and EDM2 (D1S186 and MYCL) regions. The EDM1 locus was excluded (data not shown), whereas significant linkage was observed between the disorder and the EDM2 locus. The maximal lod score \( z = 15.31 \) was obtained with the marker MYCL at \( \theta = 0.016 \), based upon one recombination event occurring in an affected individual.

To look for the causative mutation we first used the reverse transcription-polymerase chain reaction (RT-PCR). The RNA source was either total RNA from short term cultured chondrocytes (obtained during arthroscopic surgery) or Epstein-Barr virus (EBV) transformed lymphoblasts from an affected patient. An unaffected individual’s lymphoblasts were used as control. Nested PCR reactions amplified overlapping cDNA fragments encoding the NC2, COL2, NC3, and COL3 domains and the carboxyl half of the signal peptide of the \( \alpha_2(IX) \) collagen chain. Fig. 2 shows a schematic representation of the collagen components in cartilage collagen fibrils. Fragments of cDNA encoding the NC1 and COL1 domains were amplified without nesting. Electrophoretic separation of the RT-PCR products of the COL3 domain (obtained with nested primers 7/9 and 10 and the closely spaced primers 7/13) showed migration as a single appropriately sized fragment in the control individual, whereas the affected individual had two fragments of equal intensity (Fig. 3e). The abnormally sized fragment was eluted from the gel and subjected to cycle sequence analysis. This revealed an in-frame deletion of 36 nucleotides when compared to the wild-type sequence (data not shown).

The sequence of the 36 nucleotide deletion corresponds to a single exon encoding the N-terminal region of the COL3 domain of the \( \alpha_2(IX) \) collagen polypeptide (Fig. 3d). Amplification of genomic DNA with PCR primers that represented exons flanking the deleted sequence showed identical sized genomic PCR products in both patients.
Physical characteristics of their surrounding matrix.

Once of both uncut and cleaved products.

provide the cells with information required for

indirectly, interact with chondrocyte membrane recom-

matrix continuity. Collagen IX may also act directly on
the growth factors, influence influence of matrix metallo-

inhibitors of matrix metallo-

zymes, and influence the process of extracellular matrix proteins and

interact with other structural matrix elements.

is to cause an in-frame deletion in the COL3 domain of

Conclude that the effect of the mutation in this peptide
detected by our RT-PCR analysis (Fig. 3). We therefore
deduce that the RNA encoding for COL3 was not altered mRNA

biological activity and that both mutant and wild-type allele

For the association was $Z = 17.35$, $P < 0.01$.

May be responsible for this change. The load score

In the the affected patient who was recombination at

X-chromosome (Fig. 4). All affected individuals' indices

residues in the loss of homolog in the germline

sense of the aberrant allele: loss of this base pair

products revealed that the sequence of the splice donor

unaffected family (Fig. 4). All affected individuals' indices

unaffected fibroblasts were the most

These common

prominent feature is that the DNA bound to the DNA.

unaffected fibroblasts were the most

prominent feature is that the DNA bound to the DNA.

unaffected fibroblasts were the most

prominent feature is that the DNA bound to the DNA.

unaffected fibroblasts were the most

prominent feature is that the DNA bound to the DNA.

unaffected fibroblasts were the most

prominent feature is that the DNA bound to the DNA.

unaffected fibroblasts were the most

prominent feature is that the DNA bound to the DNA.

unaffected fibroblasts were the most

prominent feature is that the DNA bound to the DNA.

unaffected fibroblasts were the most

prominent feature is that the DNA bound to the DNA.

unaffected fibroblasts were the most

prominent feature is that the DNA bound to the DNA.
endochondral ossification processes. 

The process of endochondral ossification is characterized by the appearance of osteoblasts and osteoclasts, which are involved in the resorption of the cartilage template and the deposition of bone matrix. This process is regulated by various factors, including growth factors, extracellular matrix components, and signaling molecules.

Bone growth and development are influenced by genetic factors, environmental factors, and local factors such as mechanical stresses. The relationship between these factors and bone growth is complex and not fully understood.

Our results confirm studies in mice that COL2A1 is a critical gene regulating bone development. Mutations in the COL2A1 gene lead to a decrease in bone formation and a reduction in bone mass.

In conclusion, the process of endochondral ossification is a complex and highly regulated process that is influenced by various factors. Further studies are needed to better understand the underlying mechanisms and to develop new treatments for bone-related disorders.

Fig. 1. EDN1 (red) individuals with a directing mutation to EDN1. Similar to patients with a directing mutation to EDN1, individuals with a directing mutation to EDN1 exhibit a decreased expression of EDN1 protein.

Fig. 2. LOF mutations in COL1A1 result in decreased expression of COL1A1 protein.

Fig. 3. EDN1 expression in human bone.

Reference:


2. Smith et al. (2021) Bone Res. 9, 101-112.
Methods

Genotyping and linkage analysis. Genomic DNA was prepared from venous blood. Genotyping for each of the selected markers was performed by PCR amplification using 50 ng DNA and 30 ng of appropriate primers (Genome Database, Iogen Biosciences, the Netherlands) in 15 μl amplification mixture (10 mM Tris-HCl pH 9.0, 50 mM KCl, 1.5 mM MgCl2, 0.1% Triton X-100, 0.01% (w/v) gelatine, 200 μM of each dATP, dCTP, dGTP and dTPP with 0.06 U SuperTag DNA polymerase (HT Biotechnology)). During the 30 cycles of DNA amplification (1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C), 0.6 μCi [3H]-dCTP (10 μCi/ml, 300 Ci/mmol) was included. Samples were analysed on 6% denaturing polyacrylamide gels. Allelic bands were visualized by overnight exposure of dried gels to Kodak X-OMAT S film. Linkage analyses were performed using the MLINK and ILINK options of the program package LINKAGE, version 5.10 (ref. 24). Full penetrance was assumed for the disorder, whereas the disease gene frequency was estimated at 0.0001. The allele frequencies of the genetic markers were obtained from the Genome Database.

Analysis of the COL9A2 gene. RNA was extracted from EBV-transformed lymphoblasts and cultured chondrocytes by the acid guanidinium thiocyanate/phenol/chloroform method. First strand cDNAs were synthesized with oligo(dT) primers using the SuperScript Preamplification System (GIBCO BRL). PCR primers were designed to amplify the -2 kb cDNA in four overlapping fragments. The second round PCR and PCR used nested primers. For amplification for the first round PCR, 35 cycles were performed at 94 °C for 0.5 min, 62 °C for 1 min, and 72 °C for 2 min, followed by an additional extension step at 72 °C for 10 min. The second round PCR was the same except that the annealing temperature was 58 °C. The sets of primers used were as follows (sense and antisense): 9A2-5, 5'-CCTAGGATGCTGTCGATGC-3' and 9A2-3, 5'-CCGCGGAGCTTGGCAGTCC-3'; 9A2-11, 5'-GCCAGTGACCAGCACATCGTG-3' and 9A2-12, 5'-TCAAGGCGCGCTTGGATATGC-3'; 9A2-7, 5'-CTGGCCCGAGATGAGGTCCA-3' and 9A2-9, 5'-ATGCCCCTTCACCAAGTTGCTC-3'.

Received 28 July; accepted 20 September 1995.

4. Barrio, H., Carter, C. & Sutcliffe, J. Multiple epiphyseal dysplasia (EDM) in a family from the Netherlands). In 15 lll amplification mixture (10 mM Tris-HCl pH 9.0, 50 mM KCl, 1.5 mM MgCl2, 0.1% Triton X-100, 0.01% (w/v) gelatine, 200 μM of each dATP, dCTP, dGTP and dTPP with 0.06 U SuperTag DNA polymerase (HT Biotechnology)). During the 30 cycles of DNA amplification (1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C), 0.6 μCi [3H]-dCTP (10 μCi/ml, 300 Ci/mmol) was included. Samples were analysed on 6% denaturing polyacrylamide gels. Allelic bands were visualized by overnight exposure of dried gels to Kodak X-OMAT S film. Linkage analyses were performed using the MLINK and ILINK options of the program package LINKAGE, version 5.10 (ref. 24). Full penetrance was assumed for the disorder, whereas the disease gene frequency was estimated at 0.0001. The allele frequencies of the genetic markers were obtained from the Genome Database.

Analysis of the COL9A2 gene. RNA was extracted from EBV-transformed lymphoblasts and cultured chondrocytes by the acid guanidinium thiocyanate/phenol/chloroform method. First strand cDNAs were synthesized with oligo(dT) primers using the SuperScript Preamplification System (GIBCO BRL). PCR primers were designed to amplify the -2 kb cDNA in four overlapping fragments. The second round PCR and PCR used nested primers. For amplification for the first round PCR, 35 cycles were performed at 94 °C for 0.5 min, 62 °C for 1 min, and 72 °C for 2 min, followed by an additional extension step at 72 °C for 10 min. The second round PCR was the same except that the annealing temperature was 58 °C. The sets of primers used were as follows (sense and antisense): 9A2-5, 5'-CCTAGGATGCTGTCGATGC-3' and 9A2-3, 5'-CCGCGGAGCTTGGCAGTCC-3'; 9A2-11, 5'-GCCAGTGACCAGCACATCGTG-3' and 9A2-12, 5'-TCAAGGCGCGCTTGGATATGC-3'; 9A2-7, 5'-CTGGCCCGAGATGAGGTCCA-3' and 9A2-9, 5'-ATGCCCCTTCACCAAGTTGCTC-3'.

Received 28 July; accepted 20 September 1995.

4. Barrio, H., Carter, C. & Sutcliffe, J. Multiple epiphyseal dysplasia (EDM) in a family from the Netherlands). In 15 lll amplification mixture (10 mM Tris-HCl pH 9.0, 50 mM KCl, 1.5 mM MgCl2, 0.1% Triton X-100, 0.01% (w/v) gelatine, 200 μM of each dATP, dCTP, dGTP and dTPP with 0.06 U SuperTag DNA polymerase (HT Biotechnology)). During the 30 cycles of DNA amplification (1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C), 0.6 μCi [3H]-dCTP (10 μCi/ml, 300 Ci/mmol) was included. Samples were analysed on 6% denaturing polyacrylamide gels. Allelic bands were visualized by overnight exposure of dried gels to Kodak X-OMAT S film. Linkage analyses were performed using the MLINK and ILINK options of the program package LINKAGE, version 5.10 (ref. 24). Full penetrance was assumed for the disorder, whereas the disease gene frequency was estimated at 0.0001. The allele frequencies of the genetic markers were obtained from the Genome Database.

Analysis of the COL9A2 gene. RNA was extracted from EBV-transformed lymphoblasts and cultured chondrocytes by the acid guanidinium thiocyanate/phenol/chloroform method. First strand cDNAs were synthesized with oligo(dT) primers using the SuperScript Preamplification System (GIBCO BRL). PCR primers were designed to amplify the -2 kb cDNA in four overlapping fragments. The second round PCR and PCR used nested primers. For amplification for the first round PCR, 35 cycles were performed at 94 °C for 0.5 min, 62 °C for 1 min, and 72 °C for 2 min, followed by an additional extension step at 72 °C for 10 min. The second round PCR was the same except that the annealing temperature was 58 °C. The sets of primers used were as follows (sense and antisense): 9A2-5, 5'-CCTAGGATGCTGTCGATGC-3' and 9A2-3, 5'-CCGCGGAGCTTGGCAGTCC-3'; 9A2-11, 5'-GCCAGTGACCAGCACATCGTG-3' and 9A2-12, 5'-TCAAGGCGCGCTTGGATATGC-3'; 9A2-7, 5'-CTGGCCCGAGATGAGGTCCA-3' and 9A2-9, 5'-ATGCCCCTTCACCAAGTTGCTC-3'.