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A mutation in the gene encoding the α2 chain of the fibril-associated collagen IX, COL9A2, causes multiple epiphyseal dysplasia (EDM2)

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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 fingers1,2. 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)3,5, consistent with previous analyses which suggested that EDM1 and PSACH could be allelic disorders6. A second locus, EDM2, maps to chromosome 1 in the vicinity of the COL9A2 gene7. Finally, exclusion of EDM1 and EDM2 in other families suggests the existence of at least one additional locus8. 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 α2(IX) 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 reported2,10. 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 osteoarthritis 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) regions6,13. 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 θ = 0.016, based upon one recombination event occurring in an affected individual.

To look for a 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 α2(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 the more 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 α2(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

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Once of both uncleaved and cleaved products:

- Figure 4: Agarose gel electrophoresis of COL4A4 genomic frag.

The ability to amplify both mutant and wild-type allele.

For the mutation was Z = 71.35, θ = 0°.
endocondral ossification processes by specific alterations in the cellular processes by which the physical integrity of the growth plate is altered. The reduction in height of the growth plate may be due to these cellular mechanisms. The reduced cartilage thickness suggests a potential role for the COL2A1 gene in the process of endochondral ossification. Further studies are needed to elucidate the mechanism underlying this phenomenon.

The prime activity of endochondral ossification is the growth of cartilage. The process of endochondral ossification suggests a contribution of other modifying factors (e.g., FGF). The presence of multiple epiphyseal dysplasia in patients with mild osteoarthritis, however, does not appear to be of specific skeletal significance. The observations of skeletal disorders in patients with mild osteoarthritis, however, are important for understanding the role of the COL2A1 gene in the process of endochondral ossification. Further studies are needed to elucidate the mechanism underlying this phenomenon.
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 four appropriate primers (Genome Database, Isogen 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) gelatin, 200 μM of each dATP, dCTP, dGTP and dTTP) 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 was included. Samples were analysed on 6.5% denaturing polyacryamide gels. Allelic bands were visualized by overnight exposure of dried gels to Kodak X-OMAT S films. 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 COL1A2 gene. RNA was extracted from EBV-transformed lymphoblasts and cultured chondrocytes by guanidinium thiocyanate/phenol/chloroform method. First strand cDNAs were synthesized with oligo(dT) primers extracting DNA from human nucleated cells. The exon structure of the mouse a(IX) collagen gene to chromosome 11 (W. B. Saunders, Philadelphia, 1985), showed a 36 nt deletion in the lower band. To better analyze this deletion, an additional antisense primer 9A2-13, 5'-CCCTCAGCTCGAAGCTGACAGCCGCTTTGGAGCATC-3' was made and used for second round PCR with the 9A2–7 primer. PCR condition was 35 cycles at 94 °C for 0.5 min, 58 °C for 1 min, 72 °C for 2 min and additional 72 °C for 10 min at the end of cycles; 0.5 μl of [α-32P]-dCTP (10 mM, 2000 Ci/mmol) was added to the reaction. The product obtained with primers 7/13 was analysed on a 5% sequencing gel and subjected to cycle sequencing as well. For amplifying genomic DNA, two additional sense primers 9A2–14, 5'-CCTGATGAGCAGGCAATGACACAGACAGAGGTGAGCT-3' and 9A2–15, 5'-GAATGCCGCCCCCTCGAGCTAAGCTGAGAGT-3' were synthesized and the primer pairs 14/13 and 16/13 were used for PCR. 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): 14/13 and 16/13 were synthesized and the primer pairs AR36819 and AR36820 (to B. R. O.). Expert secretarial assistance for PCR reactions was done in a total volume of 50 μl containing 1 μM of each primer, 20 μM dNTPs, 0.6 μCi [3H]-dCTP with 0.06 U SuperTaq 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 was included. Samples were analysed on 6.5% denaturing polyacryamide gels. Allelic bands were visualized by overnight exposure of dried gels to Kodak X-OMAT S films. 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.

Received 28 July; accepted 20 September 1995.