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A mutation in the gene encoding the α2 chain of the fibrill-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 fingers¹-⁴. 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)⁶. Mutations in COMP have also been identified in patients with pseudoachondroplasia (PSACH)⁸, consistent with previous analyses which suggested that EDM1 and PSACH could be allelic disorders¹⁰. A second locus, EDM2, maps to chromosome 1 in the vicinity of the COL9A2 gene⁴. Finally, exclusion of EDM1 and EDM2 in other families suggests the existence of at least one additional locus¹¹. 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 previously been reported¹²,¹³. 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 statures 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/varus 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 (D18S186 and MYCL) regions⁴,⁴. 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 0 = 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 carboxy 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. 3b). 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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Figure 4 A. Agarose gel electrophoresis of COL14A1 genomic frag-

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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, Isogen Bioscience, 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 2.5 μM dTTP) with 0.06 U SuperTag DNA polymerase (HT Biotechnologies). During the 30 cycles of DNA amplification (1 min at 94 °C for 2 min at 55 °C and 1 min at 72 °C), 0.6 μCi of [3H]dCTP (10 mCi/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 films. Linkage analyses were performed using the MLINK and ILINK options of the program package LINKAGE, version 5.10 (ref. 24). Full penetration was assumed for the disorder, whereas the disease gene frequency was estimated at 0.001. 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 Pre amplification System (GIBCO BRL). PCR primers were designed to amplify the 2-1 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'-CTGGGACCTCTGCCAGTTGCTC-3' and 9A2-3, 5'-GGCGACCTCTGCCAGTTGCTC-3'. 9A2-11, 5'-GGCGACCTCTGCCAGTTGCTC-3' and 9A2-12, 5'-TCAGGACCTCTGCCAGTTGCTC-3'. 9A2-7, 5'-CTGGGACCTCTGCCAGTTGCTC-3' and 9A2-9, 5'-GGCGACCTCTGCCAGTTGCTC-3'. 9A2-13, 5'-GGCGACCTCTGCCAGTTGCTC-3' and 9A2-14, 5'-GGCGACCTCTGCCAGTTGCTC-3'. The primer pairs 9A2-11 and 9A2-12 were used for first round PCR. The product obtained with the pair 9A2-11 was analysed without further purification.

Results

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