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

Yasutere Muragaki1, Edwin C.M. Mariman2, Sylvia E.C. van Beersum2, Merja Peralä3, Jan B.A. van Mourik4, Matthew L. Warman5, Bjorn R. Olsen6 & Ben C.J. Hamel2

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 osteoarthrits associated with short stature and stubby fingers1-7. 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)8. Mutations in COMP have also been identified in patients with pseudoachondrodysplasia (PSACH)9, 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 gene11. 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 been previously 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 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 osteoarthrits with or without loose bones. 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) regions14. 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 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 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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1Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115, USA
2Department of Human Genetics, University Hospital Nijmegen, 6500 HB Nijmegen, The Netherlands
3Department of Medical Biochemistry, University of Turku, 20520 Turku, Finland
4Department of Orthopaedic Surgery, St. Joseph Hospital, 5500 MB Vél D'hoven, The Netherlands
5Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, Ohio 441067, USA

Correspondence should be addressed to B.R.O. or B.C.J.H.
PHYSICAL CHARACTERISTICS OF COLLAGEN IX

Collagen IX may also interact with other structural matrix molecules in the TGF-β growth factor family, suggesting that collagen IX may play a role in anchoring collagen fibrils to these molecules. Furthermore, collagen IX may interact with other structural matrix elements, 1-3.

Once of both uncleaved and cleaved products. This is consistent with the location of the collagen IX domain, which is in the middle of the molecule, and the fact that the cleavage site is located within the GAG domain.
endochondral ossification processes. Shortly before development in the cellular process, a specific alteration of the growth plate occurs. This alteration is caused by a deletion of COL2A1, which affects cartilage formation. The resulting lesion causes cartilage defects and subsequent growth retardation.

Our results confirm studies in mice that lack collagen IX.

Of other protein section of cartilage matrix, a domain near the C terminus of collagen IX. At the level of protein section and supermolecular assembly, it is possible that the COL7A1 mutation in this family causes a problem of supermolecular assembly; however, these proposed effects on the collagen function have little effect on polyethylene glycol. Based on these findings, the collagen IX mutation in other families collagen IX, comparable mutations can be inferred.
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 ~3-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. The sets of primers used were as follows:

- **CC-3′**: 5′-AGAGAATCCAGGAAGGCCCTG-3′
- **9A2-5′**: 5′-GGCGACCTCTGCCAGTTGCTC-3′

The primer pairs 5′/2′ were designed to amplify the ~2 kb fragment. The second round PCR used nested PCR primers. The primer pairs 9A2-7 primer. PCR condition was 35 cycles at 94 °C for 0.5 min, 62 °C for 1 min, and at 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):

- **9A2-14**: 5′-CCTGGATCCGACGGCA-3′
- **9A2-13**: 5′-GCGACGCTCCCTGGACCTC-3′

The primer pairs 5′/2′ were used for first round PCR. The product obtained with the pair 5′/12 was analysed without further amplification. The original GenBank/EMBL file (accession number M59610) has been updated to include the additional unsequenced flanking sequences. The sequence of the COL9A2 gene is deposited (H. van Mourik, J.B.A. Schaap, C. & Nolen A.J.G., unpublished observations).

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