The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/23763

Please be advised that this information was generated on 2019-10-26 and may be subject to change.
A mutation in the gene encoding the α2 chain of the fibril-associated collagen IX, COL9A2, causes multiple epiphyseal dysplasia (EDM2)

Yasuteru Muragaki1, Edwin C.M. Mariman2, Sylvia E.C. van Beersum2, Merja Perala3, Jan B.A. van Mourik4, Matthew L. Warman5, Bjorn R. Olsen1 & 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 osteoarthritis 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 pseudoachondroplasia (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 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) regions14,15. 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 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. 3). 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

---

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 Veldhoven, The Netherlands
5Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106, USA

Correspondence should be addressed to B.R.O. or B.C.J.H.
A picture of the page is not available, but the text mentions:

- "physical characteristics of their surrounding matrix..."
- "collagen IX may also direct or maintain cartilage homeostasis. Rather than interacting with other structural matrix elements..."
- "can be produced, than collagen II is produced by its own collagen cross-links to collagen II. The cartilage integrity of cartilage decreased even further. Consequently, collagen IX may be the consequence of decreased pepsin linked to EDNRB. Osseointegrations and processes are..."
- "The ability to amplify both mutan and wildep type..."

From the text, it seems there is a discussion on the role of collagen IX in maintaining cartilage homeostasis and its interaction with other structural matrix elements. The text also mentions the production of collagen IX by its own collagen cross-links to collagen II and the significance of the cartilage integrity of cartilage decreased even further. The text suggests the importance of collagen IX in maintaining cartilage integrity and highlights the ability to amplify both mutan and wildep type sequences.
endochondral ossification processes, and in particular, the production of the cellular processes by which the process of endochondral ossification is initiated. The process of endochondral ossification is initiated by the appearance of cartilage-specific factors, including specific transcription factors (e.g., Sp7) and specific matrix molecules that bind to these factors. The process of endochondral ossification is initiated by the appearance of specific transcription factors, including specific transcription factors (e.g., Sp7) and specific matrix molecules that bind to these factors. The process of endochondral ossification is initiated by the appearance of specific transcription factors, including specific transcription factors (e.g., Sp7) and specific matrix molecules that bind to these factors.
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 the 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 Biotechnology). During the 30 cycles of DNA amplification (1 min at 94 °C, 2 min at 55 °C and 1 min at 72 °C), 0.6 μCi [3H]-dTTP (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 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 0.001. The allele frequencies of the genetic markers were obtained from the Genome Database.

Analysis of the COLA2 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 (Genome Database, Isogen Bioscience, the Netherlands). Hecht, J. et al. 5'-CTCCAGGTGGTAGTGCTC-3' and 9A2-5, 5'-CTCCAGGTGGTAGTGCTC-3' were synthesized and the primer pairs 14/13 and 16/13 were used for first round PCR. The products obtained with primers 16/13 from genomic DNA were sequenced. The original GenBank/EMBL file (accession number M59610) had been updated to include the additional unpublished sequences. The general GenBank/EMBL file (accession number M59610) was used for further analysis.

The product obtained with the pair 5/2 was amplified further by PCR. The conditions were heating at 95 °C for 0.5 min, 64 °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 as were follows (sense and antisense): 9A2-5, 5'-CTCCAGGTGGTAGTGCTC-3' and 9A2-3, 3'-CAATCCCGGGCTTCCCGTCTG-3'. 9A2-11, 5'-GGCCATGACACGACACATGTC-3' and 9A2-12, 5'-TCAAGGCCCCTTGTAGGTC-3'. 9A2-7, 5'-CTGGCGAGATACGTCCT-3' and 9A2-9, 5'-AGGCCCTTCCACGCCCTGAC-3'. 9A2-1, 5'-GCGGATTTCCTGTGTCCAA-3'. The product obtained with primer pair 9A2-11 was used for first round PCR. The product obtained with the pair 11/12 was analysed without further amplification.

Acknowledgments

We thank H.H. Rupes, H.G. Brunner and E. Vasco for helpful discussions and comments. This work was supported by NIH grants AR36819 and AR36820 to B.R.O. (B.R.O.). Expert secretarial assistance was provided by Y. Fittel.

Received 28 July; accepted 20 September 1995.