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Genetic linkage analysis of 14 candidate gene loci in a family with autosomal dominant osteoarthritis without dysplasia

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Abstract

The role of various gene loci was investigated in a family in which familial osteoarthritis (FOA), with onset at an early age, is transmitted as an autosomal dominant mendelian trait. The absence of clinical and radiographic signs of dysplasia and calcium pyrophosphate deposition disease (CPDD) indicates that the basic disease process in this family is osteoarthritis (OA). Genetic linkage analysis of 14 candidate genes resulted in the exclusion of 10 important genes (COL2A1, COL9A1, COL9A2, COL11A1, COL11A2, COMP, the CPDD region, CRTL-1, CRTM, and MMP3). Other relevant genes were not informative in this family. The candidate loci previously identified in FOA and heritable skeletal disorders associated with OA are clearly not involved in the development of the primary FOA phenotype in the family investigated, indicating genetic heterogeneity.

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Osteoarthritis (OA) is a degenerative disease of the joints, characterised by degradation of the hyaline articular cartilage and remodelling of the subchondral bone with sclerosis. Genetic factors play a role in the aetiology of familial OA (FOA) with an early age of onset (20-40 year) in multiple joints.

Table 1 Candidate genes with associated disease used for linkage analysis

Gene	*Associated disease*	*Reference*
COL2A1	Several types of chondrodysplasia, FOA*, Stickler syndrome	2–7
COL9A1	OA, mild chondrodysplasia in mice	8 9
COL9A2	EDM2†	10 11
COL9A3	—	—
COL11A1	Stickler syndrome, chondrodysplasia in mice	12 13
COL11A2	Stickler/Kniest dysplasia, osteochondrodysplasias	14 15
DCN	—	—
CRTL1	—	—
COMP	PSACH‡, EDM1§	16 17
CRTM	—	—
CCAL2	Familial CPDD ‖	18
LOX	Ehlers-Danlos type IX	19
PLOD	Ehlers-Danlos type VI	20 21
MMP3	—	—

COL2A1=α1 collagen type II gene. COL9A1=α1 collagen type IX gene. COL9A2=α2 collagen type IX gene. COL9A3=α3 collagen type IX gene. COL11A1=α1 collagen type XI gene. COL11A2=α2 collagen type XI gene. DCN=decorin gene. CRTL-1=cartilage link protein gene. COMP=cartilage oligomeric protein gene. CRTM=cartilage matrix gene. CCAL2=locus of familial chondrocalcinosis. LOX=lysyl oxidase gene. PLOD=lysyl hydroxylase gene. MMP3=stromelysin I gene. *FOA=familial osteoarthritis. †EDM2=multiple epiphyseal dysplasia type 2. ‡PSACH=pseudoachondrodysplasia. §EDM1=multiple epiphyseal dysplasia type 1. ‖CPDD=calcium pyrophosphate dihydrate deposition disease.

Research into the genetic loci involved in FOA has so far mainly been focused on the type II procollagen gene (COL2A1). The COL2A1 gene encodes the major stress resisting element and the most abundant structural collagenous protein in cartilage.[1] Genetic studies of families and unrelated patients with an FOA phenotype accompanied by mild spondyloepiphyseal dysplasia (SED) have shown linkage to COL2A1 and mutations in the gene in approximately 25% of the cases.[2] However, only a few studies have focused on families where OA is the only and primary disease process without any dysplasia. Genetic studies showed positive linkage to COL2A1 in two such FOA families.[3 4] Mutation analysis of the COL2A1 gene in 45 unrelated FOA patients showed only one COL2A1 mutation.[5] Studies on other candidate genes have not been reported for FOA. Genes, however, identified in heritable skeletal disorders associated with generalised OA may also play a possible role in FOA (table 1).

We have investigated the role of 14 candidate genes for FOA in a four generation Dutch family of Jewish descent in which the pedigree included 21 persons (fig 1). Informed consent and complete medical history was obtained for all family members. The study was approved by the Medical Ethics Committee of the Academic Hospital Leiden. Physical examinations were performed on all members of the third generation and on seven out of 14 direct descendants in the fourth generation. All clinical evaluations and diagnostic decisions were made before the genetic linkage analyses.

The family included many members affected with OA with an age of onset between 20 and 40 years. Symptoms began with intermittent acute pain and swelling in one or both knee joints with subsequent development of OA in other joints. The hip was only rarely affected (table 2). Radiographic signs of chondrodysplasia, spinal dysplasia, or abnormal development of the epiphyses of the peripheral joints were absent (fig 2). Some subjects had marked Heberden nodes (table 2, fig 3). The mean ratio of upper to lower segment of affected members was 0.95 (range 0.89 to 1.02), indicating the absence of a short trunk form of dysplasia. All family members had normal stature (table 2). The diagnosis was "pure" familial OA transmitted as an autosomal dominant mendelian trait (fig 1, table 2).

Genetic linkage analysis was performed using either intragenic or closely linked

markers to the candidate genes (table 3). Genomic DNA samples of family members were collected by mouth swabs.[37] All markers, except D20S19, were analysed by genomic PCR containing α[32P]-dCTP.[38] Alleles were separated by standard electrophoresis through a denaturing polyacrylamide gel (3.5-6%) and visualised by autoradiography.[38] Alleles of marker D20S19 were analysed by Southern blotting and hybridisation with the clone pCMM20 radioactively labelled with α[32P]-dCTP.[38]

Two point lod scores between the disease phenotype of family members and the markers were calculated using MLINK from the LINKAGE package version 5.1.[39] Multipoint lod score analysis was performed using the LINKMAP program.[40] The disease locus was modelled as an autosomal dominant trait. People with clinical and radiographic evidence of OA in two or more joints were considered affected. Penetrance was modelled to rise linearly from 0% at age 15 to 100% at 40 years,

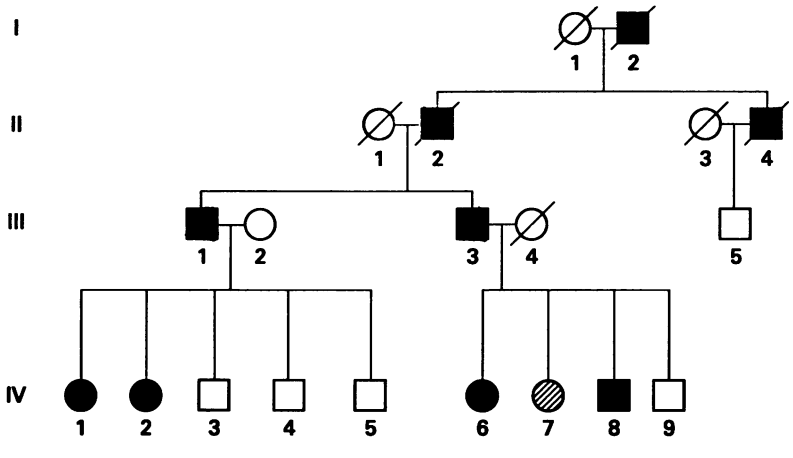

Figure 1 Pedigree of the FOA family used for linkage analysis. The hatched symbol represents diagnostic uncertainty, the filled symbols represent affected subjects.

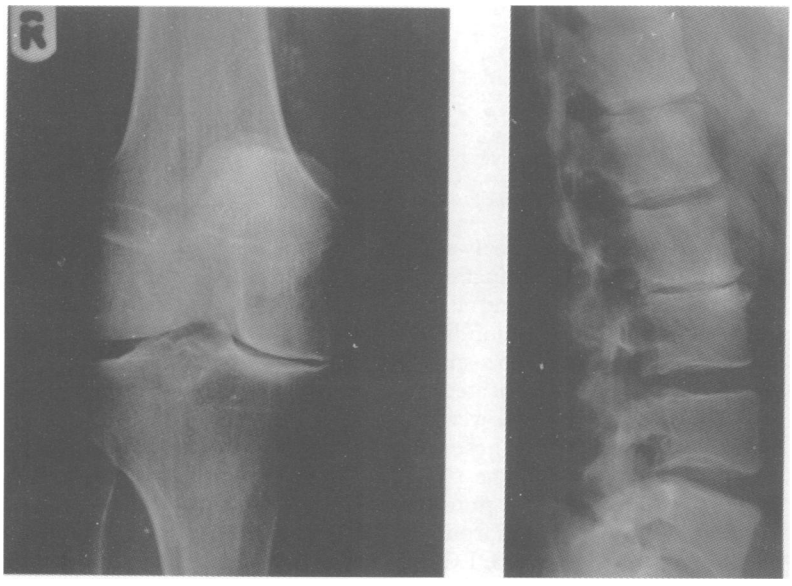

Figure 2 X rays of III.1 aged 53 years. (A) Right knee joint showing marked OA medially and chondrocalcinosis laterally, without evidence of epiphyseal dysplasia. (B) Lumbosacral spine joints (lateral view) showing osteophytosis and discus degeneration at level L2-L3. No dysplastic features.

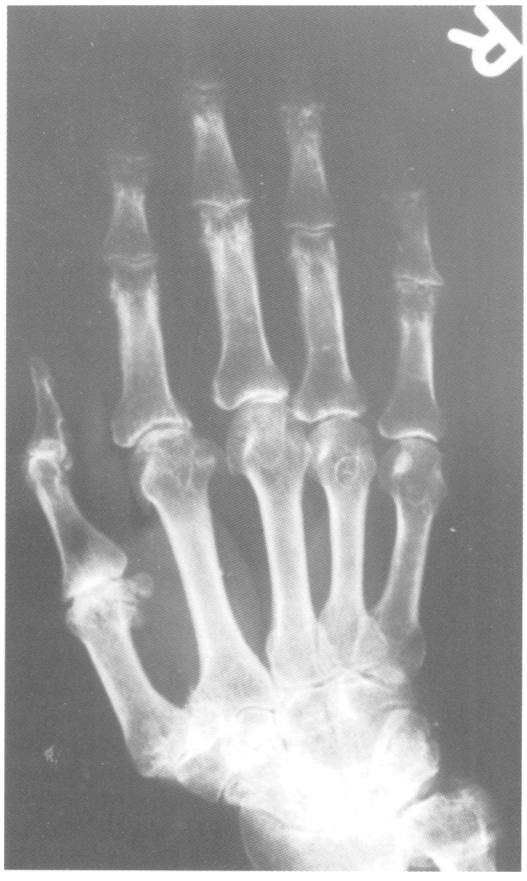

Figure 3 X ray of III.3 aged 65 years showing hand joints with marked OA in DIP, PIP, CMC1, MCP-I, and II. Heberden and Bouchard nodes are visible.

Table 2 Radiographic and clinical abnormalities in members of a family with autosomal dominant FOA

Patient (age)	*M/F*	*DIP*	*PIP*	*MCP*	*CMC1*	*Elbow*	*Shoulder*	*Hip*	*Knee*	*Foot*	*Ankle*	*CS*	*LS*	*No*	*BMI*	*Height*	*U/L*
II.2(—)	M	—	—	—	—	OA	—	N	OA,C	OA	OA	—	—	4			
III.1(73)	M	P,H	—	—	OA	OA,C	OA	—	OA,C	OA	OA	OA	OA	9	26.8	1.76	1.02
III.3(70)	M	OA,H	OA,B		OA	P	—	—	OA,C	—	OA	OA	—	9	24.3	1.64	0.96
III.5(67)	M	—	—	—	—	—	—	N	—	—	—	OA	OA	2			
IV.1(42)	F	N	N	N	OA	—	—	—	OA	—	N	OA	OA	4	26.3	1.70	0.83
IV.2(41)	F				N	N	—	—	OA,C	—	P	OA	—	3	23.4	1.68	0.95
IV.3(39)	M	—	—	—	—	—	—	—	—	—	—	—	—	0		1.76	
IV.4(37)	M	N	N	N	N	—	—	N	N	—	—	—	—	0		1.80	
IV.5(35)	M	—	—	—	—	—	—	N	N	—	—	—	—	0		1.76	
IV.6(42)	F	N	N	N	OA	—	—	N	OA	—	—	OA		3			
IV.7(40)	F	—	—	—	—	—	—	—	—	—	—	OA	—	1			
IV.8(38)	M	—		—	—	N	—	—	OA,C	OA	N	OA	N	3	27.5	1.71	0.91
IV.9(27)	M	—		—	—		—	—	—	—	—	—	—	0	22.4	1.83	0.91

M/F=male/female. DIP=distal interphalangeal joints. PIP=proximal interphalangeal joints. MCP=metacarpophalangeal joints. CMC1=first carpometacarpal joints. CS=cervical spine joints. LS=lumbar spine joints. No=number of joints affected with radiographic/clinical OA. BMI=body mass index. U/L=upper/lower segment ratio. — =no *x* ray taken, no clinical signs. OA=radiographic osteoarthritis according to Kellgren (grade 2 or higher) in one or both joints. N=radiographically normal joint on both sides (Kellgren 0 or 1). C=radiographic chondrocalcinosis. P=clinical signs of OA (bony enlargements/joint deformity/limited range of movement). H=Heberden's nodes. B=Bouchard's nodes.

Table 3 Chromosomal location of candidate genes with polymorphic markers used for linkage analysis

Gene	Location	Marker	Distance (Kosambi cM) between marker and gene	Reference
COL2A1	12q12-q13.2	VNTR	0 KcM	22
COL9A1	6q12-q14	8B1	Intragenic	23
COL9A2	1p32.3-p33	MYCL1	0.2 KcM	24 25
COL9A3	20q13.3	D20S19	5 KcM	26 27
COL11A1	1p21	7B1	Intragenic	*
COL11A2	6p21.3	TNF locus; D6S291	Map element 3.5 KcM	28–30
DCN	12q21.3-q23	Dinucleotide intron 1A	Intragenic	31
CRTL1	5q13-q14.1	Dinucleotide promoter	Intragenic	32
COMP	19q12	D19S212	0.8 KcM	29
CRTM	1p35	Dinucleotide 3' UTR	Intragenic	33
		D1S247; D1S513	Map element 2 KcM	
CCAL2	8q	D8S545	0 KcM	29
LOX	5q23.3-q31.2	RFLP exon 1 (*PstI*)	Intragenic	34
PLOD	1p36.3-p36.2	FRG (1p36.2-p36.1)	15 KcM	35
MMP3	11q22-q23	D11S35	2 KcM	36

*Personal communication, M Warman.

Table 4 Two point lod scores calculated between familial osteoarthritis (FOA) and markers within or flanking the candidate gene loci

	Recombination fraction (θ)								Exclusion
Genes	0.00	0.001	0.01	0.05	0.1	0.2	0.3	0.4	(KcM)*
COL2A1	−∞	−2.01	−1.02	−0.39	−0.17	−0.03	−0.00	−0.01	0.1
COL9A1	−∞	−1.96	−0.97	−0.31	−0.08	0.05	0.04	−0.00	0.1
COL9A2	−∞	−4.86	−2.86	−1.47	−0.88	−0.35	−0.11	−0.01	2.7
COL9A3	−∞	−1.86	−0.87	−0.20	0.04	0.18	0.15	0.06	0.1
COL11A1	−∞	−2.85	−1.85	−1.14	−0.81	−0.44	−0.20	−0.06	0.7
COL11A2	−∞	−1.81	−0.81	−0.14	0.10	0.21	0.24	0.24	0.1
DCN	0.41	0.41	0.40	0.37	0.31	0.20	0.10	0.03	—
CRTL1	−∞	−4.29	−2.31	−0.99	−0.49	−0.11	0.01	0.03	1.4
COMP	−∞	−4.90	−2.90	−1.51	−0.94	−0.41	−0.16	−0.04	2.8
CRTM	−0.01	−0.00	−0.00	0.01	0.01	0.01	0.01	0.00	—
8q	−∞	−3.38	−2.33	−1.42	−0.92	−0.40	−0.14	−0.02	1.9
LOX	0.36	0.36	0.35	0.30	0.25	0.15	0.07	0.02	—
PLOD	−∞	−5.05	−3.05	−1.63	−1.02	−0.45	−0.17	−0.04	3.3
MMP3	−∞	−4.85	−2.85	−1.47	−0.90	−0.39	−0.15	−0.04	2.7

*The excluded distance is calculated with odds of 100:1 against linkage in Kosambi centiMorgans (KcM).

based on the onset age of OA as described previously.[3] Subjects with ages of onset above 40 years were considered as phenocopies.

Lod scores and the recombination fractions yielding a lod score of −2 are shown in table 4. Since the lod scores over the genetic region covered by the COL2A1, COL9A1, COL9A2, COL11A1, CRTL-1, COMP, CPDD, and MMP3 genes are ≤ −2 (odds 100:1 against linkage), these genes were excluded from being involved in causing FOA in this family. The COL9A3, COL11A2, DCN, CRTM, LOX, and PLOD genes could not be excluded by two point linkage analysis (table 4). The COL11A2 and CRTM genes were excluded (lod scores ≤ −2, table 5) by using multipoint lod score analysis with fixed markers and known recombination fractions (table 3). For the COL9A3 and PLOD genes, the excluded region surrounding the highly polymorphic markers was too small to exclude the genes. The markers located within the DCN and LOX genes (table 3) were not informative in important meioses. These genes could, therefore, not be excluded. The COL9A3 and DCN loci are mapped in regions containing too few alternative polymorphic markers and the genetic location of the LOX and PLOD genes is not well defined.

Table 5 Multipoint lod score analysis of disease locus (1) versus map element of polymorphic markers (2 and 3)

COL11A2 1=2=3	*Lod score**	*COL11A2* 2=1=3	*Lod score**
0.500 0.035	0.00	0.000 0.035	−∞
0.400 0.035	0.05	0.007 0.028	−2.56
0.300 0.035	0.17	0.014 0.022	−2.08
0.200 0.035	0.26	0.021 0.015	−2.08
0.100 0.035	0.22	0.028 0.007	−2.26
0.000 0.035	−15.12	0.035 0.000	−∞
CRTM 1=2=3	*Lod score**	*CRTM* 2=1=3	*Lod score**
0.500 0.020	0.00	0.000 0.020	−∞
0.400 0.020	0.00	0.004 0.016	−3.83
0.300 0.020	−0.10	0.008 0.012	−3.23
0.200 0.020	−0.36	0.012 0.008	−2.88
0.100 0.020	−0.96	0.016 0.004	−2.63
0.000 0.020	−32.22	0.020 0.000	−2.43

*Computed by division of the location scores with 2 ln (10).

In summary, 10 genes were excluded from involvement in FOA in this family. Among these loci were important candidate genes involved in several heritable skeletal disorders, mild chondrodysplasia and epiphyseal dysplasia associated with early onset OA in multiple joints (COL2A1, COL9A1, COL9A2, COL11A1, COL11A2, COMP, and the CPDD region). Other possible candidate genes encoding non-collagenous structural components, or genes involved in post-translational modification and remodelling of cartilage, were also excluded (CRTL-1, CRTM, and MMP3). The COL9A3, DCN, LOX, and PLOD genes could not be excluded as the cause of FOA in our family.

We have shown once more that FOA is genetically heterogeneous and that 10 important OA candidate genes are not involved in the pathogenesis of an FOA phenotype without dysplasia. Unidentified genes may be detected in future studies of this and other families in which FOA is the primary disease process.

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