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

I Meulenbelt, C Bijkerk, F C Breedveld, P E Slagboom

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.
(J Med Genet 1997;34:1024-1027)

Keywords: linkage analysis; familial osteoarthritis; chondrodysplasia; candidate genes

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.

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

TNO-PG, Gaubius Laboratory, Department of Vascular and Connective Tissue Research, PO Box 2215, 2301 CE Leiden, The Netherlands
I Meulenbelt
C Bijkerk
P E Slagboom

Department of Epidemiology and Biostatistics, Erasmus University Medical School, Rotterdam, The Netherlands
C Bijkerk

Departments of Rheumatology and Internal Medicine, University Hospital, Leiden, The Netherlands
I Meulenbelt
F C Breedveld

Correspondence to: Dr Slagboom.

Received 5 March 1997
Revised version accepted for publication 23 May 1997

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.

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,

Pedigree

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 disc 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 (<i>Pst</i> I)	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

Genes	Recombination fraction (θ)								Exclusion (KcM) *
	0.00	0.001	0.01	0.05	0.1	0.2	0.3	0.4	
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

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).

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.

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.

We would like to thank Dr J K van der Korst, Dr P J J M Rompa, and Professor Dr J J Rasker for providing us with the x rays and medical histories of their patients, Saskia de Wildt for technical support, and Dr M Warman and Dr C J Williams for helpful discussions. This work was supported by the TNO-Health Organisation, the "Dutch League against Rheumatism" of the Netherlands, and Looscofoundation.

- 1 Prockop DJ, Kivirikko KI. Collagens: molecular biology, diseases, and potentials for therapy. *Annu Rev Biochem* 1995;64:403-34.
- 2 Williams CJ, Jimenez SA. Heritable diseases of cartilage caused by mutations in collagen genes. *J Rheumatol* 1995;22(suppl 43):28-33.
- 3 Palotie A, Väisänen P, Ott J, et al. Predisposition to familial osteoarthritis linked to type II collagen gene. *Lancet* 1989; i:924-7.
- 4 Vikkula M, Palotie A, Ritvaniemi P, et al. Early-onset osteoarthritis linked to the type II procollagen gene. *Arthritis Rheum* 1993;36:401-9.
- 5 Ritvaniemi P, Kärkkö J, Bonaventure J, et al. Identification of COL2A1 gene mutations in patients with chondrodysplasias and familial osteoarthritis. *Arthritis Rheum* 1995;38: 999-1004.
- 6 Pun YL, Moskowitz RW, Lie S, et al. Clinical correlations of osteoarthritis associated with a single-base mutation (arginine⁵¹⁹ to cysteine) in type II procollagen gene. *Arthritis Rheum* 1994;37:264-9.
- 7 Williams CJ, Rock M, Considine E, et al. Three new point mutations in type II procollagen (COL2A1) and identification of a fourth family with the COL2A1 Arg519Cys base substitution using conformation sensitive gel electrophoresis. *Hum Mol Genet* 1995;4:309-12.
- 8 Nakata K, Ono K, Miyazaki JI, et al. Osteoarthritis associated with mild chondrodysplasia in transgenic mice expressing $\alpha 1$ (IX) collagen chains with a central deletion. *Proc Natl Acad Sci USA* 1993;90:2870-4.
- 9 Fassler R, Schnegelsberg PNJ, Dausman J, et al. Mice lacking $\alpha 1$ (IX) collagen develop non-inflammatory degenerative joint disease. *Proc Natl Acad Sci USA* 1994;91:5070-4.
- 10 Briggs MD, Choi HC, Warman ML, et al. Genetic mapping of a locus for multiple epiphyseal dysplasia (EDM2) to a region of chromosome 1 containing a type IX collagen gene. *Am J Hum Genet* 1994;55:678-84.
- 11 Muragaki Y, Mariman ECM, van Beersum SEC, et al. A mutation in the gene encoding the $\alpha 2$ chain of the fibril-associated collagen IX, COL9A2, causes multiple epiphyseal dysplasia (EDM2). *Nat Genet* 1996;1:103-5.
- 12 Richard AJ, Yates JRW, Williams R, et al. A family with Stickler syndrome type 2 has a mutation in the COL11A1 gene resulting in the substitution of glycine 97 by valine in $\alpha 1$ (XI) collagen. *Hum Mol Genet* 1996;5:1339-43.
- 13 Li Y, Lacerda DA, Warman ML, et al. A fibrillar collagen gene COL11A1 is essential for skeletal morphogenesis. *Cell* 1995;80:423-30.
- 14 Brunner HG, Beersum SEC, Warman ML, Olsen BR, Ropers HH, Mariman ECM. A Stickler syndrome gene is linked to chromosome 6 near the COL11A2 gene. *Hum Mol Genet* 1994;3:1561-4.
- 15 Vikkula M, Mariman ECM, Lui VCH, et al. Autosomal dominant and recessive osteochondrodysplasias associated with the COL11A2 locus. *Cell* 1995;80:431-7.
- 16 Briggs MD, Hoffman SMG, King LM, et al. Pseudoachondroplasia and multiple epiphyseal dysplasia due to mutations in the cartilage oligomeric matrix protein gene. *Nat Genet* 1995;10:330-6.
- 17 Cohn DH, Briggs MD, King LM, et al. Mutations in the cartilage oligomeric matrix protein (COMP) gene in pseudoachondroplasia and multiple epiphyseal dysplasia. *Ann NY Acad Sci* 1996;785:188-94.
- 18 Baldwin CT, Farrer LA, Adair R, Dharmavaram R, Jimenez S, Anderson L. Linkage of early-onset osteoarthritis and chondrocalcinosis to human chromosome 8q. *Am J Hum Genet* 1995;56:692-7.
- 19 Kuivaniemi H, Peltonen L, Kivirikko KI. Type IX Ehlers-Danlos syndrome and Menkes syndrome: the decrease in lysyl oxidase activity is associated with a corresponding deficiency in the enzyme protein. *Am J Hum Genet* 1985;37:798-808.
- 20 Heikkinen J, Hautala T, Kivirikko KI, Myllylä R. Structure and expression of the human lysyl hydroxylase gene (PLOD): introns 9 and 16 contain Alu sequences at the sites of recombination in Ehlers-Danlos syndrome type VI patients. *Genomics* 1994;24:464-71.
- 21 Hyland J, Ala-Kokko L, Royce P, Steinmann B, Kivirikko K, Myllylä R. A homozygous stop codon in the lysyl hydroxylase gene in two siblings with Ehlers-Danlos syndrome type VI. *Nat Genet* 1992;2:228-31.
- 22 Berg ES, Olaisen B. Characterization of the COL2A1 VNTR polymorphism. *Genomics* 1993;16:350-4.
- 23 Warman ML, Tiller GE, Polumbo PA, et al. Physical and linkage mapping of the human and murine genes for the $\alpha 1$ chain of the type IX collagen (COL9A1). *Genomics* 1993;17:694-8.
- 24 Mäkelä TP, Hellsten E, Vesa J, Alitalo K, Peltonen L. An Alu variable poly A repeat polymorphism upstream of L-Myc at 1p32. *Hum Mol Genet* 1992;1:217.
- 25 Hellsten E, Vesa J, Heiskanen M, et al. Identification of YAC clones for human chromosome 1p32 and physical mapping of the infantile neuronal ceroid lipofuscinosis (INCL) locus. *Genomics* 1995;25:404-12.
- 26 Brewton RG, Wood BM, Ren ZX, et al. Molecular cloning of the $\alpha 3$ chain of human type IX collagen: linkage of the gene COL9A3 to chromosome 20q13.3. *Genomics* 1995; 30:329-36.
- 27 Steinlein O, Fisher C, Keil R, Smigrodzki R, Vogel R. D20S19, linked to low voltage EEG, benign neonatal convulsions, and Fanconi anaemia, maps to a region of enhanced recombination and is localized between CpG islands. *Hum Mol Genet* 1992;1:325-9.
- 28 Nedospasov SA, Udalova IA, Kuuprash DV, Turetskaya RL. DNA sequence polymorphism at the human tumor necrosis factor (TNF) locus. *J Immunol* 1991;147:1053-9.
- 29 Gyapay G, Morissette J, Vignal A, et al. The 1993-94 Génethon human genetic linkage map. *Nat Genet* 1994;7:246-339.
- 30 Martin G, Mann D, Carington M. Recombination rates across the HLA complex: use of microsatellites as a rapid screen for recombinant chromosomes. *Hum Mol Genet* 1995;4:423-8.
- 31 Briggs MD, Cohn DH. Length polymorphism within a complex dinucleotide repeat in the human decorin (DCN) gene. *Hum Mol Genet* 1993;7:1087.
- 32 Hecht JT, Wang Y, Rhodes C, Yamada Y. GT repeat polymorphism in the human proteoglycan link gene promoter region. *Nucleic Acids Res* 1991;19:6666.
- 33 Wang Y, Sadler L, Hecht JT. Polymorphic dinucleotide repeat in a cartilage matrix protein (CRTM). *Hum Mol Genet* 1992;1:780.
- 34 Csiszar K, Mariani TJ, Gosin JS, Deak SB, Boyd CD. A restriction fragment length polymorphism results in a non-conservative amino acid substitution encoded within the first exon of the human lysyl oxidase gene. *Genomics* 1993; 16:401-6.
- 35 Patel MS, Mankoo BS, Bruckell PM. A polymorphic microsatellite repeat is located close to the promoter region of the c-fgr proto-oncogene (FGR) at chromosome 1p36.2-p36.1. *Hum Mol Genet* 1992;1:65.
- 36 Litt M, Sharma V, Luty JA. Dinucleotide repeat polymorphism at the D11S35 locus. *Nucleic Acids Res* 1990;18: 5921.
- 37 Meulenbelt I, Droog S, Trommelen GJM, Boomsma DI, Slagboom PE. High-yield noninvasive human genomic DNA isolation method for genetic studies in geographically dispersed families and populations. *Am J Hum Genet* 1995; 57:1252-4.
- 38 Sambrook J, Fritsch EJ, Maniatis T. *Molecular cloning: a laboratory manual*. Vols I, II, III. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
- 39 Lathrop GM, Lalouel JM, Julier C, Ott J. Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. *Am J Hum Genet* 1985;37:482-98.
- 40 Ott J. *Analysis of human genetic linkage*. Revised ed. Baltimore: Johns Hopkins University Press, 1991.
- 41 Hanson IM, Gorman P, Lui VCH, Cheah KSE, Solomon E, Trowsdale J. The human $\alpha 2$ (XI) collagen gene (COL11A2) maps to the centromeric border of the major histocompatibility complex on chromosome 6. *Genomics* 1989;5:925-31.