Am. J. Hum. Genet. 48:518–524, 1991

Mild Spondyloepiphyseal Dysplasia (Namaqualand Type): Genetic Linkage to the Type II Collagen Gene (COL2A1)

Carron Sher,* Rajkumar Ramesar,* Robert Martell,† Ian Learmonth,‡ Petros Tsipouras,§ and Peter Beighton*

*Medical Research Council Unit for Inherited Skeletal Disorders, Department of Human Genetics, and †Provincial Laboratory for Tissue Immunology, University of Cape Town Medical School, and ‡Department of Orthopaedic Surgery, Princess Alice Orthopaedic Hospital, Cape Town; and §Department of Pediatrics and Molecular Genetics Laboratory, University of Connecticut Health Center, Farmington

Summary

Namaqualand spondyloepiphyseal dysplasia (NSED) is a mild autosomal dominant form of spondyloepiphyseal dysplasia in which changes are maximal in the femoral capital epiphyses and the vertebral bodies. The condition is present in a large multigeneration South African family, and it is clinically important by virtue of severe progressive degenerative osteoarthropathy of the hip joint, which frequently necessitates prosthetic joint replacement in adulthood. Linkage studies using molecular markers have shown that the loci for the NSED and type II collagen genes are linked (LOD score 7.98 at a recombination fraction of .00).

Introduction

Namaqualand spondyloepiphyseal dysplasia (NSED), previously referred to as "Namaqualand hip dysplasia" (Beighton et al. 1984; Learmonth and Beighton 1987), is an autosomal dominant skeletal disorder in which changes are maximal in the hip joint and the vertebral bodies. Affected persons develop progressive degenerative osteoarthropathy of the hip in childhood and usually require prosthetic joint replacement in adulthood.

In view of the predominant involvement of the vertebrae and femoral capital epiphyses, NSED could be classified in the broader group of spondyloepiphyseal dysplasias (SED). It differs, however, from the classical autosomal dominant (AD) and X-linked (XL) forms of SED by virtue of comparatively normal height and a paucity of additional stigmata.

Mutations in the gene encoding for type II (cartilage) collagen (COL2A1) have been detected in individuals from two families with classical AD SED congenita (Lee et al. 1989; Tiller et al. 1990). As type II collagen is a major constituent of epiphyseal cartilage, we have undertaken appropriate linkage studies in NSED by using protein and DNA markers. A LOD score of 7.98 at a recombination fraction (θ) of 0 indicates linkage between the NSED phenotype and the type II collagen locus.

Received September 12, 1990; revision received October 18, 1990.

Address for correspondence and reprints: Professor P. Beighton, Department of Human Genetics, University of Cape Town Medical School, Observatory 7925, Cape Town, South Africa; or Dr. Petros Tsipouras, University of Connecticut Health Center, Farmington, CT 06032.

0002-9297/91/4803-0012$02.00

Patients and Methods

The Affected Family

NSED was documented in 45 persons in 5 generations of a South African family of mixed ancestry (Beighton et al. 1984). In the initial study 19 females and 10 males had radiographic evidence of NSED. In addition, there was good evidence that 16 persons who were either deceased or unavailable for study were also affected. The mode of transmission was clearly AD (Beighton et al. 1984; Learmonth and Beighton 1987).

The affected kindred have their antecedents in Namaqualand, a remote semidesert region about 500 miles north of Cape Town—hence the designation of the disorder. The family had genetic contributions from Khoisan (Bushman and Hottentot), Cape Malay

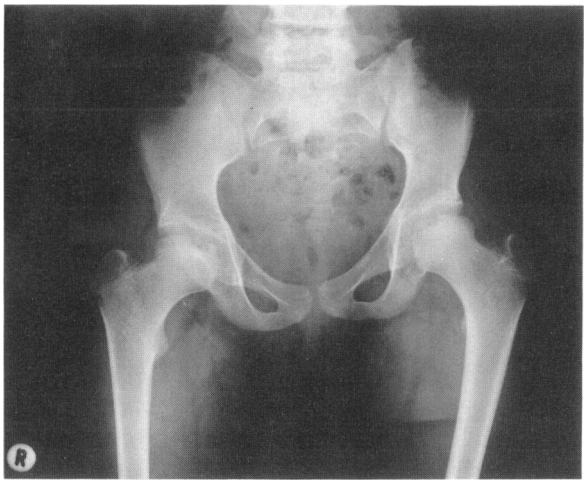

Figure 1 Anteroposterior view of hips of affected child aged 10 years, showing flattening and fragmentation of femoral capital epiphyses.

(Javanese and Sumatran), African Negro (West African and Madagascan), and Western European sources. It is uncertain whether the mutant gene was derived from one of these populations or has arisen more recently by spontaneous mutation.

The Phenotype

Affected persons experienced progressive discomfort in the hip joints, with onset in midchildhood and severe handicap by adulthood (Beighton et al. 1984; Learmonth and Beighton 1987). General health was otherwise good, and stature was essentially normal. Inconsistent mild to moderate spinal malalignment and myopia in a small proportion of affected persons were the only other significant clinical problems.

Radiographic changes were most evident in the hip joints (fig. 1). The femoral capital epiphyses displayed irregularity and alteration in texture during the first decade (fig. 2). The femoral heads became progressively misshapen and sclerotic; secondary degenerative arthropathy of the hip joints supervened in adulthood. Mild to moderate platyspondyly with end plate sclerosis and irregularity (fig. 3) were present in 60% of affected persons. The skeleton was otherwise radiologically normal, apart from inconsistent minor changes

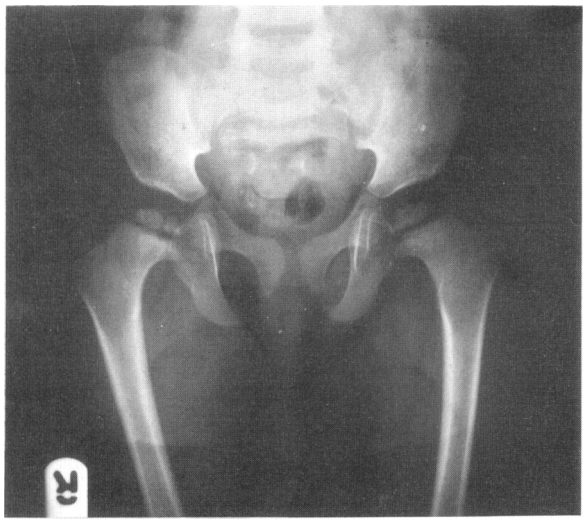

Figure 2 Anteroposterior radiograph of hips of affected adult aged 36 years. Severe secondary arthropathy has supervened.

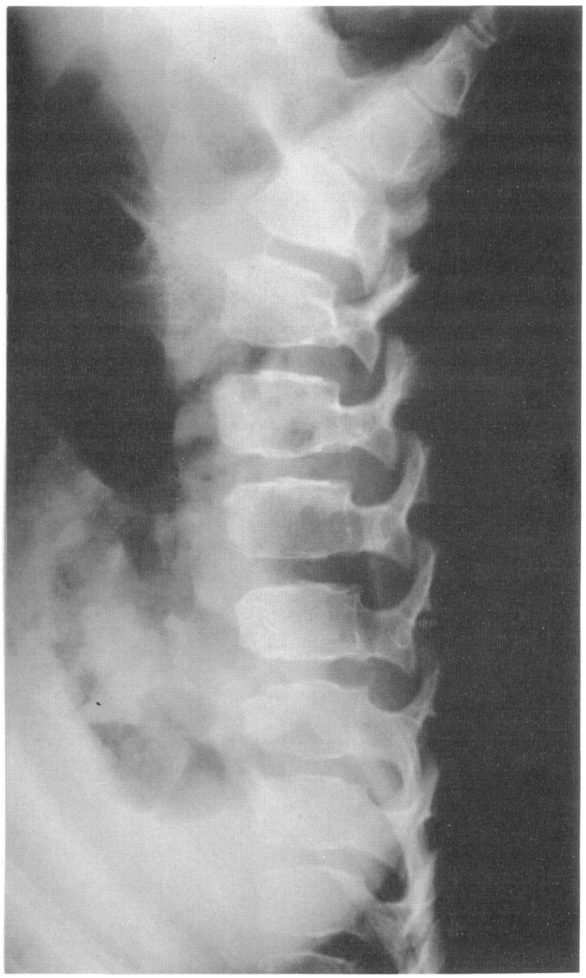

Figure 3 Lateral radiograph of spine of affected patient. The vertebral bodies are somewhat dysplastic and flattened.

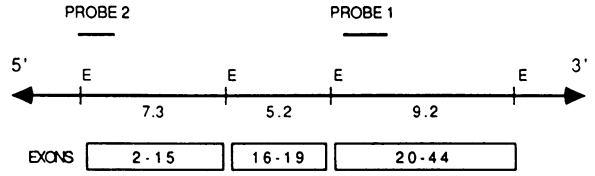

Figure 4 *Eco*RI (E) restriction map of COL2A1. Relative positions of the two probes used to detect COL2A1 RFLPs are shown.

in the femoral necks, humeral heads, and margins of the iliac crests.

Routine hematological and biochemical investigations in 10 affected persons yielded normal results. Results of cytogenetic studies in three persons were unremarkable.

Linkage Analysis

Onset of the disease was usually in the first or second decade of life. The condition is progressive, and, prior to the age of 10 years, positive clinical and radiographic diagnosis is not always possible. For this reason children aged 10 years and younger in whom there was no definite radiological evidence of the disorder were excluded from linkage analysis.

Protein Markers

Family relationships were confirmed by comparing maternal, paternal, and offspring results for each marker. During subsequent linkage analysis, one child was excluded on the basis of nonpaternity.

To investigate whether the NSED phenotype segregated with known chromosomal markers, linkage studies in the affected persons in 3 generations were carried out by R. M. in the Provincial Laboratory for Tissue Immunology, Cape Town, by using standard techniques. The following genetic markers were used: complement component 3 (C3), esterase D (ESD), glyoxalase 1 (GLO1), haptoglobin (HP), human leukocyte antigen (HLA), phosphoglucomutase 1 (PGM1), properdin factor B (BF), ABO, MNS, rhesus, and Duffy blood groups, and vitamin D–binding protein (DBP). The allele frequencies were adjusted for ethnic origin, and these values are based on established allele frequencies determined by the aforementioned laboratory.

RFLP Analysis

DNA Probes.—To investigate genetic linkage between the NSED phenotype and type II collagen, the following COL2A1 probes were used: (1) a 2.0-kb *Xho*I/*Kpn*I subclone of the 8.0-kb *Eco*RI/*Bam*HI genomic fragment of Pis2 (HcolIIc), for the *Pvu*II and *Hin*dIII RFLPs (Sangiorgi et al. 1985), and (2) a 1.6-kb *Eco*RI/*Bgl*II subclone of the 7.3-kb *Eco*RI fragment of Pis10 (HcolIIFB1), for the *Hin*fI RFLP (Strom 1988). The relative positions of the two probes on the COL2A1 gene are shown in figure 4.

DNA probe hybridization.—Nuclear DNA was extracted from peripheral blood leukocytes according to a method described elsewhere (Kunkel et al. 1977). Five micrograms of purified DNA were digested to completion with the appropriate restriction endonucleases (either *Pvu*II, *Hin*dIII, or *Hin*fI) under conditions recommended by the manufacturer. Resulting DNA fragments were separated by electrophoresis on 0.8% (*Pvu*II and *Hin*dIII) or 2% (*Hin*fI) agarose gels at 20–45 V for 24–48 h and were denatured and transferred to nylon membranes (Hybond N^+; Amersham) according to the manufacturer's instructions. Filters were prehybridized overnight in 3 × SSC, 5 × Denhardt's solution, 0.5% SDS, and 0.2 mg salmon sperm DNA/ml. The DNA probes were labeled with ^{32}P to a specific activity of 1×10^9 cpm/μg by using the random oligonucleotide primer method (Feinberg and Vogelstein 1984) and hybridized to the membrane for 48 h. Two 30-min stringency washes of 3 × SSC and 0.1% SDS, followed by two 30-min washes in 0.1 × SSC and 0.1% SDS were carried out at 65°C prior to autoradiography at 70°C for 1–10 d.

Pairwise LOD scores were calculated using the computer program LIPED (Ott 1974). The NSED gene was assumed to be AD with a penetrance of 100% and with an allele frequency of .001. Likelihoods were calculated at various θ values from .0 to .4. Confidence limits were calculated using the method recommended by Conneally et al. (1985).

Results

Linkage Analysis

A total of 41 individuals (as indicated in the pedigree shown in fig. 5) were genotyped. No linkage with the serum protein and blood group markers could be established.

RFLP Analysis

The family was tested for genetic linkage of the NSED phenotype to the gene for type II procollagen by using RFLP analysis. RFLPs for the probes, HcolIIc and HcolIIFB1, have been described elsewhere (Sangi-

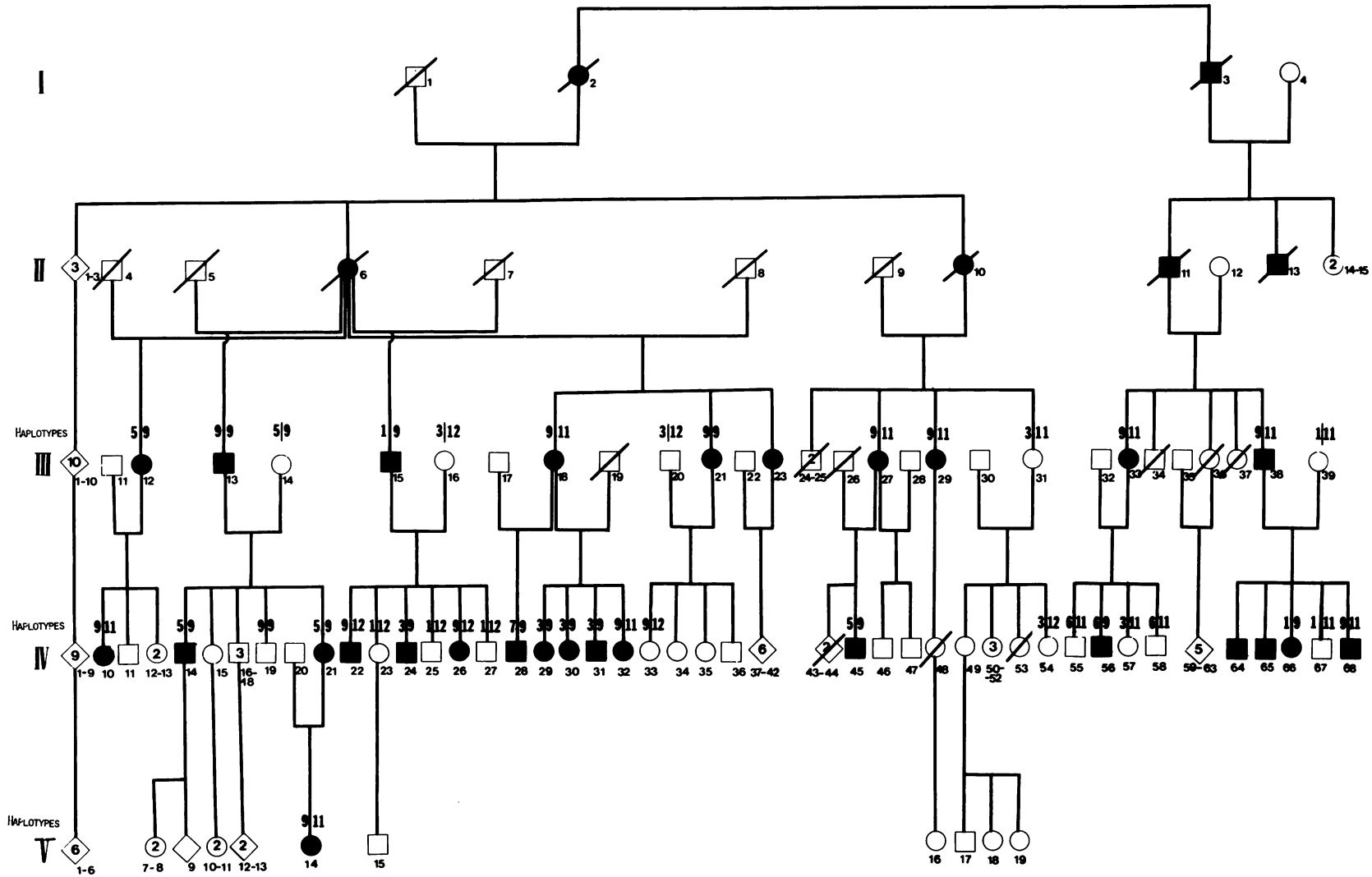

Figure 5 Haplotyped NSED pedigree. □ = unaffected male; ○ = unaffected female; ■ = affected male; ● = affected female

Table 1

Allele Frequencies for Mixed-Ancestry Population of South Africa

Probe, Restriction Endonuclease (no. of chromosomes analyzed), and Allele (kb)	Frequency
HcolIIC:	
*Pvu*II (54):	
1 (3.3)	.44
2 (1.6)	.56
HcolIIFB1:	
*Hin*dIII (40):	
1 (14)	.60
2 (7)	.40
*Hin*fI (40):	
1 (2.1)	.22
2 (1.7)	.18
3 (1.0 and .7)	.60

orgi et al. 1985; Strom 1988). No new polymorphisms were detected with these two probes in the previously unstudied South African mixed-ancestry population. In addition, all three polymorphic systems were in Hardy-Weinberg equilibrium. The frequencies of RFLPs in a control sample of normal unrelated individuals are shown in table 1.

The *Pvu*II, *Hin*dIII, and *Hin*fI RFLPs were informative in this family. Using the genotypes obtained with each enzyme/probe/RFLP combination allowed manually constructed haplotypes to be assigned unambiguously to each analyzed member of the pedigree. The possible haplotype combinations were designated as shown in table 2.

Table 2

Haplotype designations fromRFLP Allele Data

	RFLP Alleles		
Haplotype Designation	*Hin*fI	*Hin*dIII	*Pvu*II
1	1	1	1
2	2	1	1
3	3	1	1
4	1	2	1
5	2	2	1
6	3	2	1
7	1	1	2
8	2	1	2
9	3	1	2
10	1	2	2
11	2	2	2
12	3	2	2

Table 3

Lod Scores for NSED and COL2A1 Haplotypes at Various θ Values

θ	LOD Score
.0	7.98
.05	7.26
.10	6.51
.20	4.93
.30	3.21
.40	1.42

Seven of these haplotypes (1, 3, 5, 7, 9, 11, and 12) were present in individuals of this pedigree. The haplotypes of analyzed individuals are indicated on the pedigree (fig. 5). It was evident that the NSED phenotype was segregating with haplotype 9 (genotype 2,1,3).

The LOD scores for NSED and the COL2A1 haplotypes at various θ values are shown in table 3. The maximum likelihood estimate of θ was .0, with confidence limits of .0–.07.

Discussion

Phenotypic and pedigree data indicate that NSED is an AD chondrodysplasia which can be categorized with the SED group of disorders by virtue of predominant involvement of the hip and spine. NSED differs from the well-defined classical forms of SED in which stunted stature is a major feature, and it may represent a private syndrome.

As the cartilaginous epiphyses contain abundant fibrils made up of type II collagen, this substance could well be involved in the pathogenesis of conditions—notably SED, spondyloepimetaphyseal dysplasia (SEMD), and related disorders—in which the epiphyses are predominantly affected (Horton et al. 1985). It is of interest to note the similarity of the NSED phenotype to the mild chondrodysplasia with osteoarthritis recently reported elsewhere (Ala-Kokko et al. 1990; Knowlton et al. 1990). A single basepair mutation converted the codon for arginine at position 519 to cysteine, and it was found in individuals affected with early progressive osteoarthritis. Biochemical defects of type II collagen have been identified in SED and SEMD by Murray et al. (1989). Lee et al. (1989) reported a genomic deletion in one allele spanning the middle of intron 47 to the 5′ splice site of intron 48 of the COL2A1 gene, resulting in the excision of 36 amino acids from the

α1(II) chain. Tiller et al. (1990) reported on a tandem duplication of 45 bp within exon 48, resulting in the addition of 15 amino acids to the triple-helical domain of the α1(II) chain. Abnormalities of type II collagen have been found in a number of chondrodysplasias, including type II achondrogenesis-hypochondrogenesis (Eyre et al. 1986; Godfrey and Hollister 1988; Godfrey et al. 1988). Palotie et al. (1989) reported on the linkage of familial osteoarthritis to the type II collagen gene. In addition, linkage between the phenotype and RFLPs associated with the COL2A1 gene has been demonstrated in affected persons in 4 generations of a family with AD SED congenita (Anderson et al. 1990*a*). Francomano et al. (1987) reported genetic linkage of Stickler syndrome to the COL2A1 locus. These disorders have significant phenotypic and radiologic similarities, lending credence to the "phenotypic family" concept proposed by Spranger (1985). The varying clinical features and natural history of each disorder probably reflect different pathogenetic mechanisms. A possible explanation for these variations is that the location and the nature of the α1(II) chain defect may differ in each of the previously mentioned phenotypes.

Similar genetic linkage investigations were negative in a large family with a mild form of SED (Anderson et al. 1990*b*). These observations support the contention that AD SED is probably heterogeneous, and they also indicate that the COL2A1 gene is a likely candidate in other conditions in the broader SED category.

The LOD score of 7.98 which was obtained in our investigation indicates that the NSED phenotype is associated with defects in or very near the COL2A1 gene. The pathogenesis is still uncertain, but we are hopeful that investigation of both type II collagen and mRNA from cultured chondrocytes of affected individuals will provide pertinent information. The predominant femoral head involvement in NSED is similar to that in other disorders of uncertain etiology—notably Perthes disease, Meyer arthropathy, Upington bone disease, Beukes arthropathy, and primary degenerative arthropathy of the hip. The observation that the COL2A1 gene is involved in NSED has clear implications for the investigation of these conditions.

The linkage relationship in the South African family with NSED will permit presymptomatic diagnosis. The approach is of practical significance for the clinical management of the asymptomatic potentially affected child who is too young to display the characteristic radiological changes. Antenatal diagnosis by amniocentesis will also be possible; but, in view of the nonlethal nature of the clinical manifestations, it is uncertain whether patients would choose to exercise this option of terminating an affected pregnancy.

Acknowledgments

Our research was supported by grants from the South African Medical Research Council, the Mauerberger Foundation, the Harry Crossley Fund, and University of Cape Town Staff Research Fund and by NIH grant AD-22610 and Coles Family Foundation support to P.T. We are grateful to Marlene Goedhart for her technical assistance.

References

Ala-Kokko L, Baldwin CT, Moskowitz RW, Prockop DJ (1990) Single base mutation in the type II procollagen gene (COL2A1) as a cause of osteoarthritis associated with mild chondrodysplasia. Proc Natl Acad Sci USA 87: 6565–6568

Anderson IJ, Goldberg RB, Marion RW, Upholt WB, Tsipouras P (1990*a*) Spondyloepiphyseal dysplasia congenita: genetic linkage to type II collagen (COL2A1). Am J Hum Genet 46:896–901

Anderson IJ, Tsipouras P, Sher C, Ramesar R, Martell R, Beighton P (1990*b*) Spondylopiphyseal dysplasia, mild autosomal dominant type is not due to primary defects of type II collagen. Am J Med Genet 37:272–276

Beighton P, Christy G, Learmonth ID (1984) Namaqualand hip dysplasia: an autosomal dominant entity. Am J Med Genet 19:161–169

Conneally PM, Edwards JH, Kidd KK, Lalouel J-M, Morton NE, Ott J, White R (1985) Report of the Committee on Methods of Linkage Analysis and Reporting. Cytogenet Cell Genet 40:356–359

Eyre DR, Upton MP, Shapiro FD, Wilkinson RH, Vawter GF (1986) Nonexpression of cartilage type II collagen in a case of Langer-Saldino achondrogenesis. Am J Hum Genet 39:52–67

Feinberg AP, Vogelstein B (1984) A technique for radiolabelling DNA restriction fragments to a high specific activity. Anal Biochem 137:266–267

Francomano CA, Lieberfarb RM, Hirose T, Maumenee IH, Streeter EA, Meyers DA, Pyeritz RE (1987) The Stickler syndrome: evidence for close linkage to the structural gene for type II collagen. Genomics 1:293–296

Godfrey M, Hollister DW (1988) Type II achondrogenesis-hypochondrogenesis: identification of abnormal type II collagen. Am J Hum Genet 43:904–913

Godfrey M, Keene DR, Blank E, Hori H, Sakai LY, Sherwin LA, Hollister DW (1988) Type II achondrogenesis-hypochondrogenesis: morphologic and immunohistopathologic studies. Am J Hum Genet 43:894–903

Horton WA, Chou JW, Machado MA (1985) Cartilage col-

lagen analysis in the chondrodystrophies. Coll Relat Res 5:349–354

Knowlton RG, Katzenstein PL, Moskowitz RW, Weaver EJ, Malemud CJ, Pathria MN, Jiminez SA, et al (1990) Genetic linkage of a polymorphism in the type II procollagen gene (COL2A1) to primary osteoarthritis associated with mild chondrodysplasia. N Engl J Med 322:526–530

Kunkel LM, Smith KD, Boyer SH, Borgaonkar DS, Wachtel SS, Miller OJ, Breg WR, et al (1977) Analysis of human Y chromosome variants. Proc Natl Acad Sci USA 74: 1245–1249

Learmonth ID, Beighton P (1987) Namaqualand hip dysplasia: orthopaedic implications. Clin Orthop 218:142–147

Lee B, Vissing H, Ramirez F, Rogers D, Rimoin D (1989) Identification of the molecular defect in a family with spondyloepiphyseal dysplasia. Science 244:978–980

Murray LW, Bautista J, James PL, Rimoin DL (1989) Type II collagen defects in the chondrodysplasias. 1. Spondyloepiphyseal dysplasias. Am J Hum Genet 45:5–15

Ott J (1974) Estimation of the recombination fraction in human pedigrees: efficient computation of the likelihood for human linkage studies. Am J Hum Genet 26:588–597

Palotie A, Ott J, Elima K, Cheah K, Vaisanen P, Ryhanen L, Vikkula M, et al (1989) Predisposition to familial osteoarthritis linked to type II collagen gene. Lancet 1:924–927

Sangiorgi FO, Benson Chanda V, deWet WJ, Sobel ME, Tsipouras P, Ramirez F (1985) Isolation and partial characterization of the entire pro collagen gene. Nucleic Acids Res 13:2207–2224

Spranger JW (1985) Pattern recognition in bone dysplasias. In: Papadatos CJ, Bartsocas CS (eds) Endocrine genetics and genetics of growth. AR Liss, New York, pp 315–342

Strom CM (1988) A three allele restriction fragment length polymorphism within the human COL2A1 gene. Nucleic Acids Res 16:9077

Tiller GE, Rimoin DL, Murray LW, Cohn DH (1990) Tandem duplication within a type II collagen gene (COL2A1) exon in an individual with spondylophyseal dysplasia. Proc Natl Acad Sci USA 87:3889–3893