European Journal of Human Genetics (1998) 6, 251–256

http://www.stockton-press.co.uk/ejhg

ORIGINAL PAPER

Assignment of gene responsible for progressive pseudorheumatoid dysplasia to chromosome 6 and examination of *COL10A1* as candidate gene

Hatem El-Shanti[1,2], Jeffrey C Murray[4,5], Elena V Semina[4], Kenneth H Beutow[6], Titia Scherpbier[6] and Jamil Al-Alami[2,3]

Departments of [1]Pediatrics [2]Medical Technology and [3]Biochemistry, Jordan University of Science and Technology, Irbid, Jordan
Departments of [4]Pediatrics and [5]Biological Sciences, University of Iowa, USA
[6]Fox Chase Cancer Center, Philadelphia, USA

Progressive pseudorheumatoid dysplasia is an autosomal recessive skeletal dysplasia with radiographic changes in the spine similar to Spondyloepiphyseal *dysplasia tarda* and clinical, though not radiographic resemblance to rheumatoid arthritis. About two-thirds of the reported patients are of Arabic and Mediterranean origin which reflects the relative high incidence in this population. We performed homozygosity mapping utilising the DNA pooling approach to map progressive pseudorheumatoid dysplasia to a chromosomal region on the long arm of chromosome 6. We examined a possible candidate gene in the same region of linkage, namely *COL10A1*, for alterations in this disorder. We did not identify any mutations in our family, but did not totally exclude *COL10A1* gene from being the disease-causing gene.

Keywords: pooling; homozygosity mapping; pseudorheumatoid dysplasia; collagen type X

Introduction

Progressive pseudorheumatoid dysplasia (PPD) (Spondyloepiphyseal *dysplasia tarda* with progressive arthropathy (SEDT-PA) or progressive pseudorheumatoid arthropathy of childhood (PPAC), MIM 208230) is an inherited skeletal dysplasia with radiographic changes in the spine similar to Spondyloepiphyseal *dysplasia tarda* with clinical, though not radiographic, resemblance to rheumatoid arthritis.[1] It is a progressive, non-inflammatory chondropathy affecting primarily the articular cartilage with characteristic skeletal abnormalities notably in the spine.[2] A review of the published reports clearly identifies its autosomal recessive inheritance and its relative high occurrence among Arabs and other Mediterranean populations.[3] The prevalence of consanguinity among Jordanians makes it possible to identify inbred families with rare recessive disorders, such that each family is independently large enough for linkage analysis.[4,5] In one family, individuals with a rare recessive disease are likely to have inherited both copies of the mutated gene from a common ancestor, thus allowing homozygosity mapping by the DNA pooling approach.[6,7] In this study we used this strategy

Correspondence: Hatem El-Shanti, P.O. Box: 3211, Irbid 211-10, Jordan. Tel: 962-2-295111 ext: 3775; Fax: 962-2-295123; E-Mail: hatem@just.edu.jo
Received 13 August 1997; revised 23 December 1997; accepted 29 December 1997

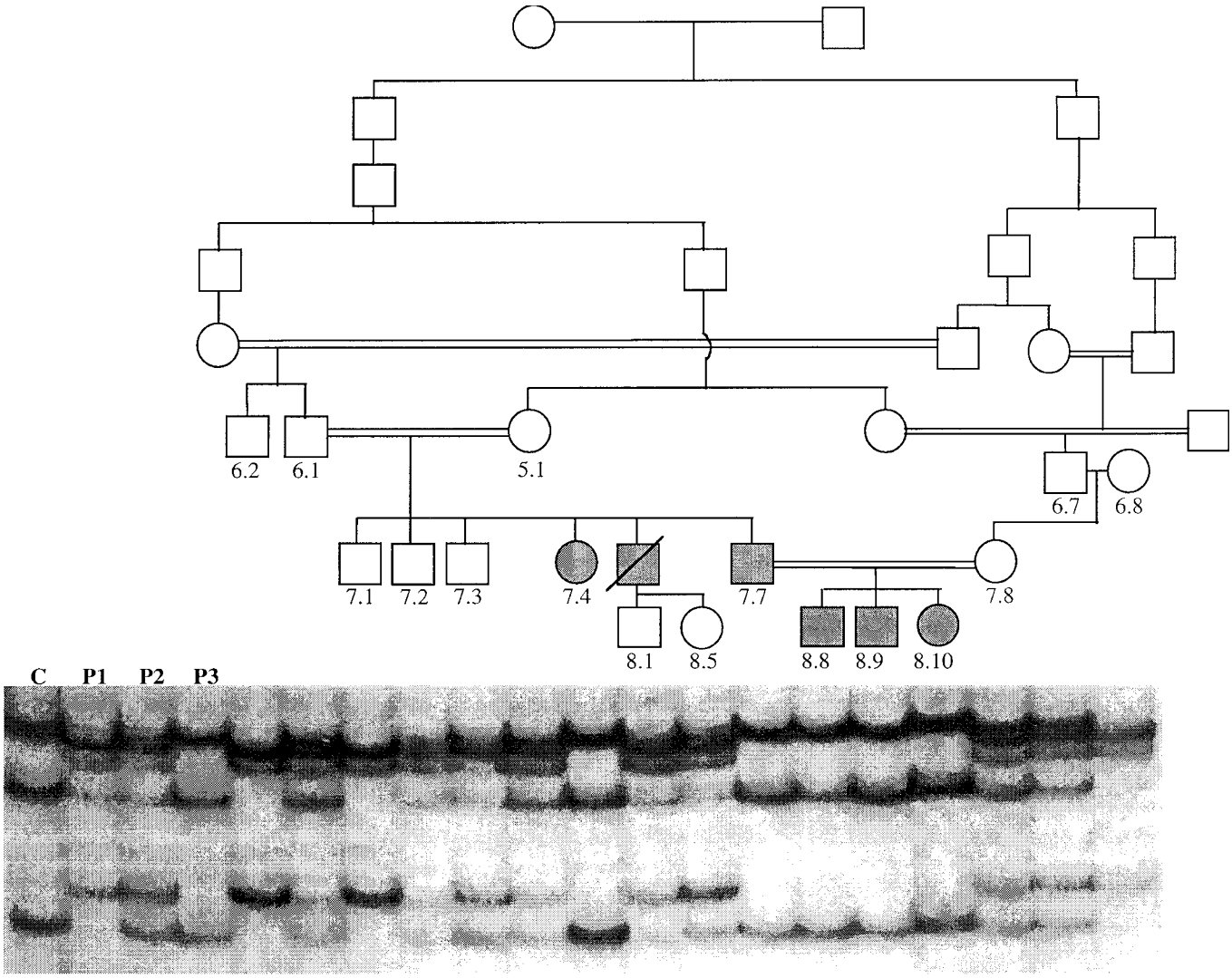

Figure 1 *The pedigree of the family used for linkage. There are four inbreeding loops. The solid symbols are for affected individuals. The bottom half shows the* COL10A1 *gene polymorphism (poly 2) with alleles shown by SSCP analysis followed by silver-staining. C = random control; P_1 = pool containing unaffected relatives; P_2 = pool containing carriers; P_3 = pool containing affecteds.*

to map the gene causing PPD to a locus on chromosome 6q in one inbred family from Jordan (Figure 1). Linkage to chromosome 6q raised the possibility that *COL10A1* might be the disease locus, but we did not identify mutations within this gene in our single family.

Materials and Methods

Pooling of DNA

DNA was extracted from dried filter card blood spots using the protocol of Descartes *et al*.[8] DNA concentrations were determined by spectrophotometric readings at OD_{260}. After the initial concentration measurement, the samples were diluted to 20 ng/μl and a second spectrophotometric reading was obtained to confirm the new DNA concentration. All 16 samples were amplified using primers of three randomly chosen STRP markers to test the quality and concentration of DNA. Equimolar amounts of samples were pooled in three groups:

1) siblings of affecteds or parents and siblings of carriers (7.1; 7.2; 7.3; 6.2; 6.7; 6.8);

2) the five obligate carriers in the pedigree who are parents or normal offspring of affecteds (5.1; 6.1; 7.8; 8.1; 8.5);

3) the five affecteds (7.4; 7.7; 8.8; 8.9; 8.10).

Genotyping

Primers for about 350 tri and tetranucleotide tandem repeat markers covering the whole genome and spaced at about 10 cM were made available to us through CHLC.[9–11]

Amplification of these markers was performed with 40 ng (2 μl) of template DNA, whether pooled or individual DNA, in an 8.4 μl PCR reaction mixture containing 1.25 μl of PCR buffer (100 mM Tris-HCl, pH 8.8; 500 mM KCl; 15 mM MgCl2; 0.01% w/v gelatin), 200 μM each of dATP, dCTP, dGTP and dTTP, 2.5 pmol of each forward and reverse primer and 0.25 U of *Taq* polymerase. The reaction mixture was subjected to 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s. Products were analysed on 6% denaturing polyacrylamide gels (7.7 M urea). The polyacrylamide gels were silver-stained using the protocol of Bassam *et al*.[12] The amplification and digestion of amplified products for the COL10A1 restriction-site polymorphism, reported in the literature, were done according to the authors' conditions.[13] Restriction products were separated on 1% agarose gel and stained with ethidium bromide. Primers (EX 2F and EX 2.1R) (Table 1) encompassing the COL10A1 polymorphic one base change (C to T; Thr 27 to Met) (poly 2) polymorphism were designed and then synthesised using phosphoramidite chemistry. The amplification was done similar to the genotyping. Amplified products were run on a fan-cooled MDE gel for SSCP analysis at 6 W for 14 h and then silver-stained.

Statistical analysis

Linkage was tested with the LOD score method using the program FASTLINK 3.0. This package requires the breaking of inbreeding loops prior to the analysis. Autosomal recessive inheritance with complete penetrance was assumed. We used a disease gene frequency of 10^{-3} and equal marker allele frequencies. We varied the marker allele frequency by using frequencies obtained from the CEPH database, as well as arbitrary allele frequencies. A LOD score of 3 or greater was used as the criterion for significance. The variation in the marker allele frequency was meant to test the strength of the significant LOD scores. The reference genetic map used for the analysis was generated by CHLC from combined CEPH data.[9] Multipoint linkage analysis could not be done due to the extensive inbreeding loops.

Table 1 Sequence of primers used for *COL10A1* amplification

Name	*Sequence*
EX 1 F	GGAGCTTAAGCTCAGGGTAA
EX 1 R	CCAAGCAAGTTAACATGGGA
EX 2 F	CTCATCTGTGAAACATGAGA
EX 2.1 R	GTTGGGTAGTGGGCCTTTTA
EX 2 R	GGGCTAATTCAGAAGTTGGA
EX 3.1 F	AACCATCCCCTTCTATGGGT
EX 3.1 R	GGAGCACCATATCCCATTTC
EX 3.2 F	TTTCCTGGAGAAAAGGGTGC
EX 3.2 R	CATCGAGACCTGGTTTTCCT
EX 3.3 F	AGGCCCTAAAGGTGAGACAG
EX 3.3 R	GGTATGGGAGTTCCTATTGC
EX 3.4 F	AGGAATGCCTGTGTCTGCTT
EX 3.4 R	TAGATTAGCTCTGTGGGGTG

F: Foward, R: Reverse.

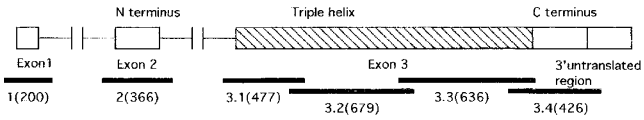

Figure 2 COL10A1 *gene organisation. The dark lines are the six amplicons produced by the primers shown in Table 2 for SSCP analysis and direct sequencing. The numbers between parenthesis denotes the length of the amplicon in base pairs.*

COL10A1 sequencing

The genomic sequence of *COL10A1* gene was obtained from GeneBank (accession number X98568). Six pairs of overlapping primers were designed producing 6 amplicons (Table 1) covering the three exons of COL10A1 gene, the splice sites and the presumed TATA box (Figure 2) and then synthesised using phosphoramidite chemistry. Amplification was performed with 200 ng (10 μl) of template DNA (from an affected carrier and normal individuals) in a 50 μl PCR reaction mixture, using similar conditions as outlined above under genotyping. The sizes of the products were verified on 1% agarose gel and recovered using a gel extraction kit (QIAGEN, Valencia, CA, USA). The extracted band was used for direct sequencing in both directions. The sequences generated were examined carefully for any difference in bases among the affected, carrier and normal individuals. A subset of products was also analysed by SSCP as described above. When the amplicon was longer than 200 bp it was cut with one or more restriction enzymes to produce fragments suitable for SSCP analysis.

Results

Linkage to chromosome 6

In order rapidly to screen for markers linked to the disease in this family, we constructed three pools each with a comparable number of individual samples.[6,7,14,15] A genome-wide screen using the three pools was started with two pools as controls. After using 140 markers covering chromosomes 1 to 6, six markers showed reduction in the number of alleles in the affected pool. Three of the markers were located within a region of 20 cM on 6q. Accordingly, individual samples were genotyped with these markers, as well as five others from the same area. Two markers were not informative in this family, and linkage was tested by 2 point lod score method for the remaining six markers. Linkage was found with markers D6S1021 and ATA56D06 with a lod score greater than 3 at a recombination fraction of zero (Table 2).

All variations in marker allele frequency gave a significant lod score, mostly even higher than the results shown in Table 2. The telomeric boundary of the region of linkage is marked by D6S1023 (Figure 3). One marker, D6S1270, centromeric to the region of linkage showed a crossover event in two of the five affecteds

Table 2 Two-point lod scores of chromosomes 6 markers with PPD locus

	Recombination fraction						
Locus	*0*	*0.01*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*
D6S1056	2.39	2.34	2.1	1.81	1.23	0.7	0.25
D6S1021	3.3	3.22	2.91	2.51	1.74	1.03	0.43
ATA56D06	3.58	3.5	3.2	2.8	2.01	1.23	0.52
D6S1023	–∞	1.92	2.3	2.22	1.72	1.1	0.49
D6S1040	–∞	–0.59	0.54	0.82	0.76	0.46	0.15
Poly 1	1.72	1.67	1.47	1.21	0.74	0.34	0.08
Poly 2	2.35	2.28	2	1.67	1.03	0.48	0.11

(results not shown) and was thus considered the centromeric boundary of the region of linkage. Pairwise analysis of the two flanking markers, using CEPH family genotypes produced a critical region of about 12.9 cM on chromosome 6q16-22 between D6S1270 and D6S1023.[9]

Examination of COL10A1 gene

The gene encoding collagen type X (*COL10A1*) became a candidate gene by virtue of its location within the same chromosomal region. In addition, its role in the endochondral ossification process supports its candidacy. Two-allele, restriction-site polymorphisms reported to be within the *COL10A1* gene were used to test for linkage but only one (poly 1) was informative in this family.[13] This *COL10A1* intragenic variant demonstrated concordant segregation with the phenotype in this family without evidence of recombination. A reported polymorphism in the second exon of *COL10A1* gene (C to T; Thr 27 to Met) (poly 2), demonstrable by SSCP analysis was used to test for linkage also indicating concordant segregation (Figure 1) (Table 2).[16,17]

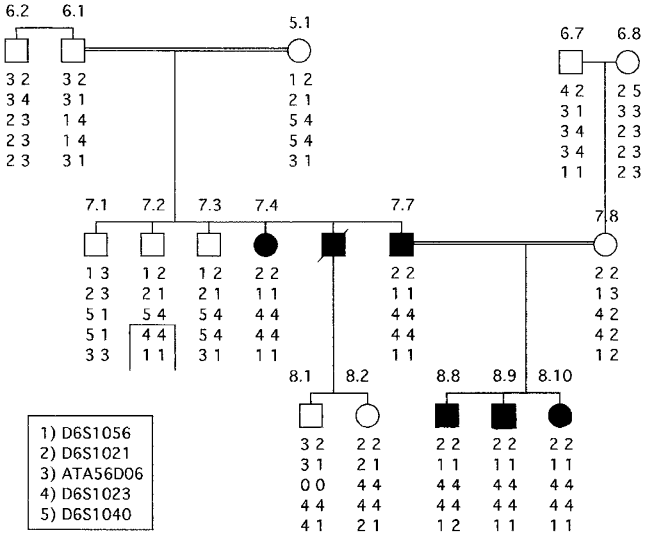

Figure 3 *A partial pedigree of the family which includes all genotyped individuals. The genotyping data for the five listed markers are shown as a haploptype. The open box is the telomeric boundary of the region of linkage. Note that affected individuals are homozygous for the same haplotype*

Screening by both direct sequencing and SSCP analysis, the three exons, 10–20 base pairs of intronic splice sites and the promotor region of the *COL10A1* gene for mutations in an affected member of this family failed to detect any deletions or point mutations (Figure 2).

Discussion

The localisation of the PPD locus to chromosome 6q is supported by three observations. The first is the significant lod score with two microsatellite markers (D6S1021 and ATA56D06) mapped to 6q. The second is that affected individuals are homozygous for all tested polymorphisms in this region while obligate carriers are heterozygous for the haplotype. The third observation is concordant segregation between the disease locus and polymorphisms within *COL10A1*, which has been localised by two different methods (somatic cell hybrids and *in situ* hybridisation) to the same region on 6q.[18,19]

In instances where a recessive disorder occurs in an inbred family, an efficient DNA pooling strategy can be used as a quick screening tool for genomic loci which are identical by descent. This strategy significantly reduces the number of amplification reactions and gel lanes needed to establish linkage. In this study, we used pools from two separate populations (unaffected relatives and obligate carriers) as controls to demonstrate efficiently the reduction in the number of alleles across the pools. Three individual false positive markers were easily recognised as false as nearby flanking markers did not show a reduction in the number of alleles. The true positive results were present in a cluster. This mapping project utilised, to our knowledge, the smallest number of individuals in each pool.

Although the basic molecular defect behind PPD is unknown, we previously speculated that alterations in the cartilage specific collagen types IX or XI or both may be implicated.[3] The gene encoding type X collagen (*COL10A1*) became a candidate gene for this disorder by virtue of its location to the same chromosomal region.[18,19] In addition several observations about collagen type X strengthens its candidacy. First, the restricted and transient expression of type X collagen in

hypertrophic chondrocytes suggests its role in the endochondral ossification process.[20] Although there is no direct evidence, the clinical and radiographic pictures of PPD suggest an alteration in the endochondral ossification process.[3] Secondly collagen type X null mice, examined grossly till six months old and histologically not later than 3 weeks, show normal long bone growth with occasional cervical spine abnormality.[21] The clinical picture of PPD includes normal long bone length and spine abnormalities similar to the collagen type X null mouse. Although *COL10A1* has been excluded as the causative gene in few other skeletal dysplasias,[22] a quantitative disturbance in the gene expression reflecting on the endochondral ossification process is consistent with the PPD phenotype and its autosomal recessive mode of inheritance.

We failed to detect any mutation in the three exons, 10–20 base pairs of intronic splice sites and the promotor region of the *COL10A1* gene in this family. However, this does not totally exclude the *COL10A1* gene from being the disease locus, as mutations might lie within introns or in the 3' untranslated region. In addition, mutations in regulatory regions not close to the genomic sequence of the gene might be the cause of this disorder in this family. Screening sporadic cases or patients from other unrelated families for mutations might be helpful in assigning PPD to the *COL10A1* gene.

Acknowledgements

We wish to thank the family members who participated readily in this study. We wish to thank Molly Wise and Bonnett Ludwig for their technical assistance and Nancy Newkirk for the help with the construction of the Figures. This study has been supported in part by a generous donation from The Tamari-Sabbagh Foundation and by the UNESCO/TWAS genome fellowship program (HES). This study has been also supported by NIH grant P50HG00835 (JCM).

References

1 Wynne Davies R, Hall B, Ansell MB: Spondyloepiphyseal dysplasia tarda with progressive arthropathy – a 'new' disorder. *J Bone Joint Surg* 1982; **64B**: 442–445.
2 Spranger J, Albert C, Schilling F, Bartsocas C, Stoss H: Progressive pseudorheumatoid arthritis of childhood (PPAC): a hereditary disorder simulating rheumatoid arthritis. *Eur J Pediatr* 1983; **140**: 34–40.
3 El-Shanti H, Omari HZ, Qubain HI: Progressive pseudorheumatoid dysplasia: report of a family and review. *J Med Genet* 1997; **34**: 559–563.
4 Al-Salem M, Rawashdeh N: Consanguinity in Jordan: prevalence and pattern. *J Biosoc Sci* 1993; **25**: 553–556.
5 Khoury SA, Massad D: Consanguineous marriage in Jordan. *Am J Med Genet* 1992; **43**: 769–775.
6 Arnheim N, Strange C, Erlich H: Use of pooled DNA samples to detect linkage disequilibrium of polymorphic restriction fragments and human disease: studies of the HLA class II loci. *Proc Natl Acad Sci USA* 1985; **82**: 6970–6974.
7 Sheffield VC, Carmi R, Kwitek-Black A *et al*: Identification of a Bardet-Biedl syndrome locus on chromosome 3 and evaluation of an efficient approach to homozygosity mapping. *Hum Mol Genet* 1994; **3**: 1331–1335.
8 Descartes L, Huang Y, Zhang YH *et al*: Genotypic confirmation from the original dried blood specimens in a neonatal hemoglobinopathy screening program. *Pediatr Res* 1991; **31**: 217–221.
9 Murray JC, Beutew KH, Weber JL *et al*: A comprehensive human linkage map with centimorgan density. *Science* 1994; **265**: 2049–2054.
10 Sheffield VC, Weber JL, Buetow KH *et al*: A collection of tri and tetranucleotide repeat markers used to generate high quality, high resolution human genome-wide linkage maps. *Hum Mol Genet* 1995; **4**: 1837–1844.
11 Yuan B, Valske D, Weber JL, Beck J, Sheffield VC: Improved set for short-tandem repeat polymorphisms for screening the human genome. *Am J Hum Genet* 1997; **60**: 459–460.
12 Bassam BJ, Caetano-Anolles G, Gresshoff PM: Fast and sensitive silver-staining of DNA in polyacrylamide gels. *Anal Biochem* 1991; **196**: 80–83.
13 Rash B, Sweetman W, Sykes B *et al*: Seven polymorphisms at the *COL10A1* locus. *Hum Mol Genet* 1994; **3**: 1032.
14 Nystuen A, Benke PJ, Merren J, Stone EM, Sheffield VC: A cerebellar ataxia locus identified by DNA pooling to search for linkage disequilibrium in an isolated population from the Cayman Islands. *Hum Mol Genet* 1996; **5**: 525–531.
15 Scott DA, Carmi R, Elbedour K, Yosefsberg S, Stone EM, Sheffield VC: An autosomal recessive nonsyndromic-hearing-loss locus identified by DNA pooling using two inbred Bedouin kindreds. *Am J Hum Genet* 1996; **59**: 395–391.
16 Sweetman WA, Rash B, Sykes B *et al*: SSCP and segregation analysis of the human type X collagen gene (*COL10A1*) in heritable forms of chondrodysplasia. *Am J Hum Genet* 1992; **51**: 841–849.
17 Reichenberger E, Beier F, Lu Valle P, Olsen GR, von der Mark K, Berting WM: Genomic organization and full-length cDNA sequence of human collagen X. *FEBS lett* 1992; **311**: 305–310.
18 Thomas JT, Cresswell CJ, Rash B *et al*: The human collagen X gene: complete primary translated sequence and chromosomal localization. *Biochem J* 1991; **280**: 617–623.
19 Apte S, Mattei MG, Olsen BR: Cloning of human alpha-1(X) collagen DNA and localization of the *COL10A1* gene to the q21-q22 region of human chromosome 6. *FEBS Lett* 1991; **282**: 393–396.

20 Thomas JT, Boot-Handford RP, Grant ME: Modulation of type X collagen gene expression by calcium glycerophosphate and levamisole: implications for a possible role for type X collagen in endochondral bone formation. *J Cell Sci* 1990; **95**: 639–648.

21 Rosati R, Horan GSB, Pinero GJ *et al*: Normal long bone growth and development in type X collagen null mice. *Nat Genet* 1994; **8**: 129–135.

22 Wallis GA, Rash B, Sykes B *et al*: Mutations within the gene encoding the alpha-1 (X) chain of type X collagen (*COL10A1*) cause metaphyseal chondrodysplasia type Schmid but not several other forms of metaphyseal chrondrodysplasia. *J Med Genet* 1996; **33**: 450–457.