The gene for pycnodysostosis maps to human chromosome 1cen–q21

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Pycnodysostosis (OMIM 265800) is an autosomal recessive skeletal disorder first described by Maroteaux and Lamy[1] that is characterized by short stature, increased bone density, delayed closure of cranial sutures, loss of the mandibular angle, dysplastic clavicles, dissolution of the terminal phalanges of the hands and feet, dental abnormalities and increased bone fragility. Patients have a typical appearance secondary to prominence of the calvarium, smallness of the facial features, prominent nose and micrognathia. The French painter, Henri de Toulouse Lautrec (1864–1901), is believed to have had the disorder[2]. Although more than 100 cases have been reported, we are aware of only two large consanguinous pedigrees in which the pycnodysostosis disorder segregates[3–5]. We have studied the segregation of the pycnodysostosis phenotype in a large consanguinous Mexican pedigree[6], the clinical features of which are very similar to those described in the Arab pedigree studied by Edelson *et al.*[3] Here, we report linkage for the pycnodysostosis phenotype in the 1cen–q21 region of human chromosome 1, and discuss candidate genes for this skeletal disorder.

In order to map the pycnodysostosis locus in this large consanguinous pedigree, we used a pooling strategy[7] in which DNA from affected individuals is pooled and genotyped. Using a total of 363 genetic markers, we compared the allelic ladders produced with the allelic ladders from a pool of unaffected heterozygote parents genotyped with the same markers. We noticed a reduction in the complexity of the number of alleles observed in the affected pool for markers *D1S1595* and *D1S534*. Genetic linkage analysis in the 24 members of this family confirmed linkage for marker *D1S1595* with a lod score of 4.11 at θ=0.05. The maximum lod score for *D1S534* was 2.05 at θ=0.08. Additional markers were typed in the region of *D1S303*, producing confirmation of linkage (Table 1). The multipoint lod score was calculated with markers positioned in the Généthon map[8]. The markers and distances used for the multipoint analysis were: *D1S514*–(0.06)–*D1S305*–(0.02)–*D1S303*–(0.03)–*D1S506*–(0.14)–*D1S194*–(0.03)–*D1S196*–(0.01)–*D1S431*. The multipoint calculation was performed without the inclusion of consanguinity loops in order to facilitate the analysis, and as a result the peak lod score was Z_{max}=3.60 at *D1S303*, smaller than the peak two point score which was calculated with the consanguinity loops (Fig. 2). Haplotype analysis in the affected individuals places the pycnodysostosis gene in a 6 cM interval between markers *D1S514* and *D1S305*.

Macrophage colony stimulating factor (MCSF), a growth factor which maps to the pericentromeric region of chromosome 1, stimulates colony formation of osteoclasts, the cells responsible for resorption and remodeling of bone. Osteoclasts are of macrophage lineage and function by dissolving bone: this process of bone resorption and regeneration is repeated constantly and allows for the continuous bone remodeling. In the similar osteopetrotic phenotype in the *op/op* mouse, an insertion at nucleotide 262 of *Csfm* gene, the homologue of human *MCSF*, results in the generation of a stop codon 21 basepairs downstream, which in turn results in a truncation of the product of the *Csfm* gene[9]. We have used the method of single strand conformation polymorphism (SSCP) analysis, in order to search for mutations in the gene that could explain the phenotype. Our analysis revealed a polymorphism within exon 6 at the fragment that is alternatively spliced and contains two asparagine-linked glycosylation sites. The polymorphism, however, did not co-segregate with the pycnodysostosis phenotype and was also seen in the parents of the CEPH reference families. These results exclude *MCSF* as the defective gene in pycnodysostosis. Other possible candidate genes in the region include the family of the S100 calcium binding proteins, which includes the calcyclin gene.

The linkage reported here for the pycnodysostosis phenotype will set the stage for the molecular investigation

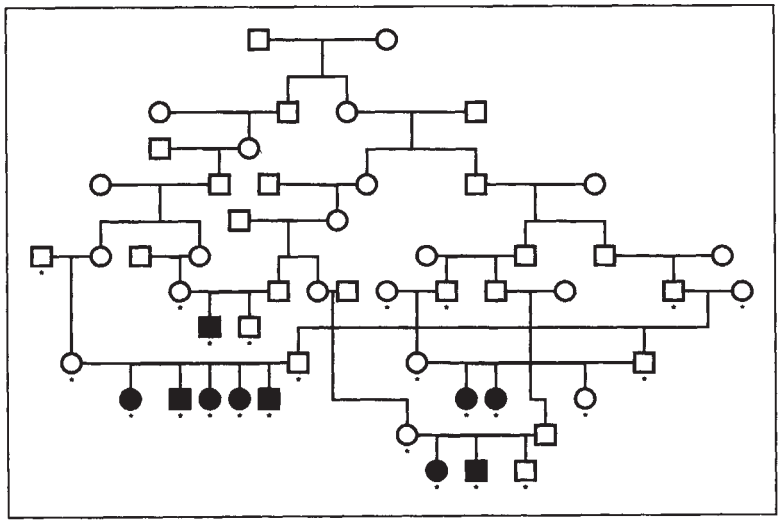

Fig. 1 Pedigree of the Mexican pycnodysostosis family. *, samples used in the study.

Table 1 Two point lod scores between chromosome 1 markers and the pycnodysostosis locus

	Recombination fraction (θ)								
Locus	0.00	0.01	0.05	0.10	0.20	0.30	0.40	Z_{max}	θ
D1S534	−∞	1.10	1.96	2.04	1.62	1.02	0.43	2.05	0.084
D1S1595	−∞	3.29	4.10	3.96	3.04	1.92	0.86	4.11	0.059
D1S514	−∞	2.13	3.10	3.12	2.52	1.65	0.77	3.16	0.075
D1S305	1.92	3.29	3.56	3.30	2.45	1.48	0.59	3.57	0.041
D1S303	3.37	3.26	3.09	2.86	2.08	1.21	0.47	3.36	0.001
D1S506	−∞	2.14	3.04	3.07	2.50	1.65	0.73	3.11	0.075
D1S194	−∞	−0.41	1.14	1.48	1.28	0.78	0.32	1.49	0.118
D1S196	−∞	−3.75	−1.38	−0.50	0.04	0.10	0.03	0.10	0.300
D1S433	−∞	−0.82	1.40	1.97	1.90	1.33	0.64	2.05	0.130
D1S431	−∞	−2.70	−0.47	0.30	0.70	0.59	0.27	0.68	0.220

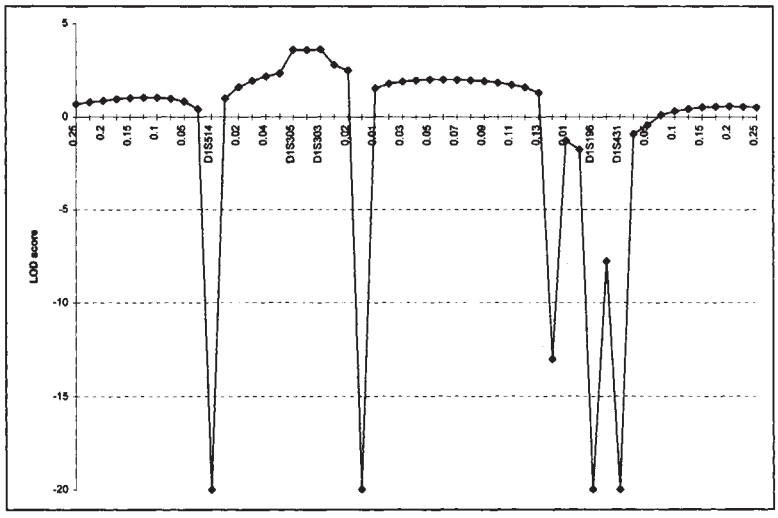

Fig. 2 Multipoint lod score graph between chromosome 1 markers and the pycnodysostosis locus.

of a unique disorder of osteogenesis, which is also of a general interest given the notion that this was the affliction of the famous french painter Toulouse Lautrec.

Methods

Clinical sample. A large Mexican pedigree was identified through the Hospital Infantil de Mexico where the proband was referred for further investigation for short stature (Fig. 1). The diagnosis of pycnodysostosis was made on the following clinical signs: 1, frequent fractures; 2, short stature; 3, frontal prominence; 3, persistence of open fontanelles; 4, facial hypoplasia; 5, small mandible; 6, dental malocclusion; 7, increased bone density; 8, parental consanguinity.

Genetic marker analysis. PCR was performed as follows: 60 ng genomic DNA were used as template with 80 ng each oligonucleotide primer, 0.6 U of *Taq* Polymerase 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, 200 µM of each dGTP, dATP, dTTP, 2.5 µM dCTP and 10 µCi of α ^{32}P dCTP, in a final reaction volume of 15 µl. PCR was performed in a Techne MW-2 microplate thermocycler as follows: denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min. The cycle was repeated 30 times with a final extension at 72 °C for 10 min. 2 µl of the reaction mix were electrophoresed on a 6% PAGE sequencing gel, using M13mp18 sequence ladders as a sizing marker and the products were visualized by autoradiography. The gels were exposed at −70 °C without prior drying. Exposure times ranged from 3–16 h.

Linkage analysis. Two point linkage analysis was peformed using the MLINK program of the LINKAGE package. Multipoint analysis was performed between two markers and the disease locus using the program LINKMAP[10] on a Sparcstation 10 computer. For the multipoint analysis the pedigree was broken up to seven individual core families in order to facilitate the analysis. Disease frequency was set to 0.001 and allele frequencies were set as described in Genome Data Base.

SSCP studies. PCR primers were developed from the published sequence for the human *MCSF* gene[11]. Primer sequences for part of exon 6 and between nts 961 and 1200 were: MCSFX8 5'–CCA GAC CCA GCA ACT TCC TCT–3' and MCSFX8.1 5'–TGG AGG GGT TGC TGA GGC CCT–3'. The size of the expected PCR product was 240 bp. For SSCP analysis the PCR samples were run on a 0.5× HydroLink™ Gel (J.T. Baker), at 4 °C at a constant power of 40 W for 3 h.

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