Am. J. Hum. Genet. 65:441–447, 1999

Localization of a Gene for Familial Patella Aplasia-Hypoplasia (PTLAH) to Chromosome 17q21–22

Massimo Mangino,[1] Otto Sanchez,[2] Isabella Torrente,[1] Alessandro De Luca,[1] Francesca Capon,[1] Giuseppe Novelli,[1] and Bruno Dallapiccola[1]

[1]Dipartimento di Biopatologia e Diagnostica per Immagini, Università di Roma Tor Vergata and Istituto CSS-Mendel, Rome; and [2]Unidad de Genetica, Escuela de Medicina, Universidad de Oriente, Ciudad Bolivar, Venezuela

Summary

Patella aplasia-hypoplasia (PTLAH) is a rare genetic defect characterized by congenital absence or marked reduction of the patella. PTLAH can occur either as an isolated defect or in association with other malformations, and it characteristically occurs in the nail-patella syndrome and in some chromosome imbalances. We report the first evidence of linkage for isolated PTLAH in an extended Venezuelan family. After exclusion of the candidate chromosome regions where disorders associated with PTLAH have been mapped, a genomewide scan was performed that supported mapping of the disease locus within a region of 12 cM on chromosome 17q22. Two marker loci (D17S787 and D17S1604) typed from this region gave maximum LOD scores >3. Accordingly, multipoint analysis gave a maximum LOD score of 3.39, with a most likely location for the disease gene between D17S787 and D17S1604. Sequencing of the *noggin* gene, a candidate mapping between these markers, failed to reveal any mutation in affected subjects.

Introduction

Patella aplasia-hypoplasia (PTLAH [MIM 168860]) is a rare genetic defect characterized by congenital absence or marked reduction of the patella bone. PTLAH can occur either as an isolated defect or in association with other malformations. Familial isolated PTLAH has been reported in only a few pedigrees, showing an autosomal dominant inheritance (Kutz 1949; Bernhang and Levine 1973; Braun 1978). Association of PTLAH with other defects, including coxa vara and tarsal synostosis, malformed pelvic girdle, and upper femora aniridia, has been observed (Goeminne and Dujardin 1970; Mirkinson and Mirkinson 1975; Scott and Taor 1979). Aplastic or hypoplastic patella occurs in distinct disorders, including Coffin-Siris syndrome, Kuskokwin syndrome, and trisomy 8 syndrome (Petajan et al. 1969; Wright 1970; Cassidy et al. 1975; Fineman et al. 1975; Silengo et al. 1979; Lucaya et al. 1981; Sujansky et al. 1981; Gorlin 1990). At present, a single gene (*LMX1B*) coding for a protein involved in the patella development has been cloned (Dreyer et al. 1998). This protein is a member of a diverse family of regulatory proteins characterized by the presence of two zinc-finger structures and a homeodomain (Curtiss and Heilig 1998). Mutations of the *LMX1B* gene have been detected in patients affected by nail-patella syndrome (NPS [MIM *161200]), a pleiotropic disorder presenting with dysplastic nails, absent or hypoplastic patella, exostoses of the ilia, dysplasia of the elbows, and, in some cases, nephropathy (Beals and Eckhardt 1969; Dreyer et al. 1998; McIntosh et al. 1998). We have investigated a four-generation family, segregating an autosomal dominant, isolated PTLAH and localizing the gene responsible for this defect to chromosome 17q21–22, by genomewide scanning.

Subjects and Methods

Family Recruitment

The investigated family is of Venezuelan ancestry and includes 26 members affected by isolated PTLAH (fig. 1). Each family member who agreed to participate in the present study was examined. Individuals were scored as affected if they had clinical and radiographic evidence of bilateral absent patella. Thus, clinical status was determined unequivocally for all participating family members. The study was approved by the institutional review boards of the Tor Vergata (Rome) and Oriente (Ciudad Bolivar) Universities, and appropriate informed consent was obtained for all subjects.

Received February 22, 1999; accepted for publication May 28, 1999; electronically published July 13, 1999.

Address for correspondence and reprints: Dr. Giuseppe Novelli, Cattedra di Genetica Umana, Università di Roma Tor Vergata, Facoltà di Medicina e Chirurgia, Ed. E-Nord Pt.terra, Via di Tor Vergata 135, 00133 Roma, Italy. E-mail: novelli@med.uniroma2.it

0002-9297/99/6502-0020$02.00

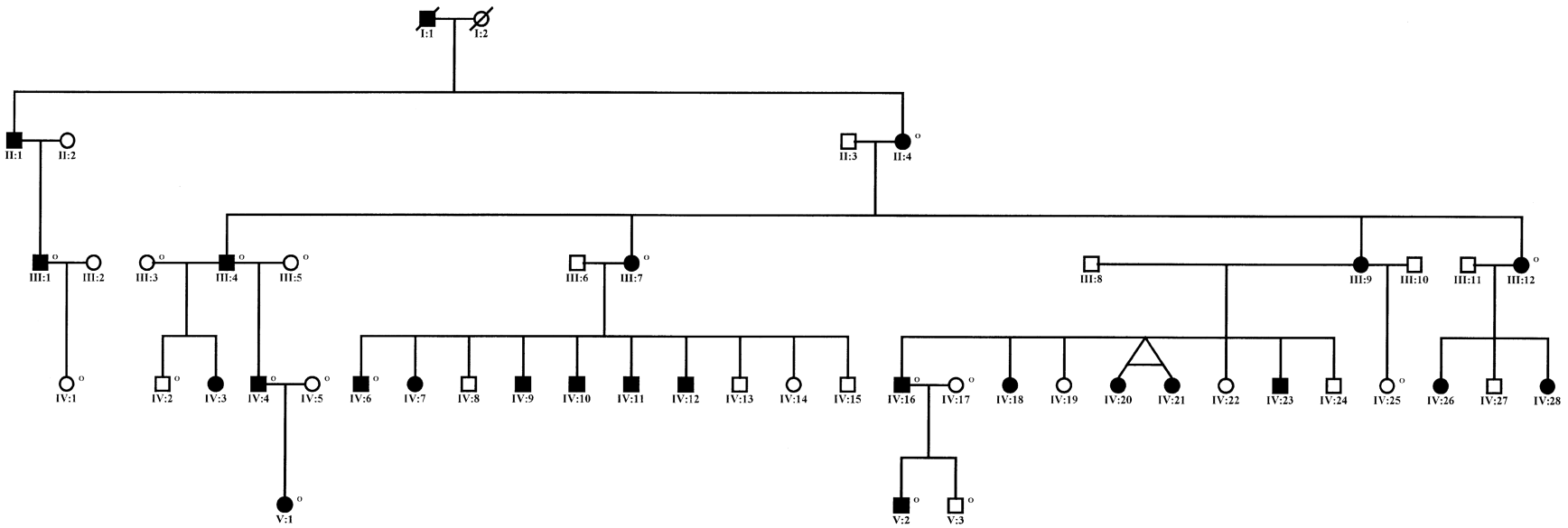

Figure 1 Pedigree of the initial branch of the kindred studied. Unblackened circles and squares indicate members who are unaffected. Blackened circles and squares indicate members reported to be affected. A degree symbol (°) indicates tested individuals.

Genotyping

Genomic DNA was extracted from the whole blood according to a standard protocol, quantified spectrophotometrically, and used at a concentration of 50 ng/μl. Markers D9S112, D9S315, D9S159, D9S195, PAX 6 (amplified by intragenic primers B509–B507), and HUMTH01 were selected on the basis of their demonstrated high LOD scores with respect to NPS and aniridia (Jordan et al. 1992; McIntosh et al. 1997). Chromosome 8q markers (D8S270, D8S276, D8S269, D8S272, D8S65, D8S53, and D8S44) were selected from the Généthon database (Weissenbach et al. 1992). Genome scanning was performed with 358 microsatellite markers loci from ABI PRISM Linkage Mapping Set (PE Applied Biosystems) at a distance of ~10 cM. PCR was performed with 50 ng of DNA in a 15-μl reaction mixture containing 1.5 μl buffer (100 mM Tris HCl, pH 8.3, 500 mM KCl), 1.5 μl $MgCl_2$ (25 mM), 1.5 μl dNTPs mix (2.5 mM), 1 μl primer mix (5 μM), and 0.6 U of AmpliTaq Gold (PE Applied Biosystems). PCR products were analyzed on a model 310 automated fluorescent DNA sequencer (PE Applied Biosystems), a four-color detection system. One microliter of PCR reaction mixture was combined with 20 μl of formamide and 0.5 μl of a fluorescent size marker (TAMRA GS-500; PE Applied Biosystems). Each sample was run for 30 min. During electrophoresis, the fluorescence detected in the laser scanning region was collected and stored with GENESCAN collection software (version 3.1; PE Applied Biosystems). The fluorescence data collected during the run were analyzed automatically by the GENESCAN analysis program (PE Applied Biosystems) at the end of each run. Each marker was examined by the GENOTYPER program (version 2.0; PE Applied Biosystems) to analyze inheritance patterns and to prepare the allele labels for export to linkage applications.

Linkage Analysis

Linkage analysis was performed with the LINKAGE 5.1 computer program package (Lathrop and Lalouel 1984). Two-point LOD scores between the disease gene and each marker were calculated by means of the MLINK program (see Lathrop and Lalouel 1984). The phenotype was coded as a fully penetrant autosomal dominant trait with a disease allele frequency of .0001. Equal recombination frequencies for men and women were assumed. The order of the markers' loci and their recombination distances used for multipoint linkage analysis were based on the Généthon linkage map (Weissenbach et al. 1992; Gyapay et al. 1994; Dib et al. 1996). Multipoint analysis was performed by the VITESSE computer program (O'Connell and Weeks 1995).

Candidate Gene Analysis

The *noggin* cDNA sequence (Valenzuela et al. 1995) was used to design primers NOG1 (corresponding to nt 770–790) and NOG5 (corresponding to nt 1550–1531), which amplify a 780-bp product containing the entire coding sequence (included in a single exon) plus 400 bp of the 5′ UTR. After gel purification, each sample was sequenced with the Thermo-Sequenase cycle-sequencing kit (Amersham Life Sciences) with IRD-41–labeled primers (Maceratesi et al. 1996), according to the manufacturer's instructions. Sequences were then run on a LI-COR 4000L automated sequencer (LI-COR).

Results

Clinical Presentation

The family included 26 members affected by isolated PTLAH (fig. 1). The proband (III-7) was clinically evaluated after a routine medical examination for an unrelated disease. On clinical inspection, bilateral absent patella was suspected, which was confirmed by radiographic study (fig. 2). The patient indicated that several other family members presented similar discomfort at knee articulation (fig. 2). Direct clinical and radiographic evaluations were performed in 15 affected subjects, whereas isolated PTLAH was assumed also to be present in 11 additional persons on the basis of anamnestic in-

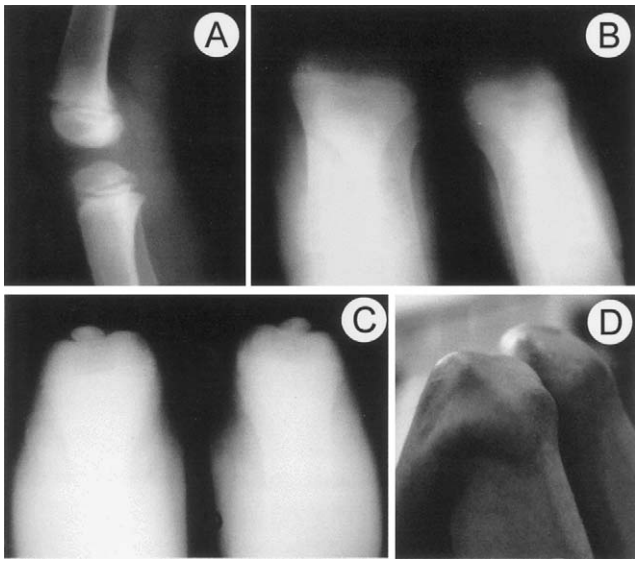

Figure 2 Radiographic screening of the knees of three affected members (IV-6, V-2, and IV-12). *A*, Lateral projection (view) of the right knee of an 8-year-old boy (IV-6) with absence of the ossification center of the patella. *B*, Axial projection (view) for the patella of a 10-year-old (V-2) subject in which the ossification center of the patella is absent bilaterally. *C*, *D*, Evidence of patella hypoplasia in a 25-year-old subject (IV-12).

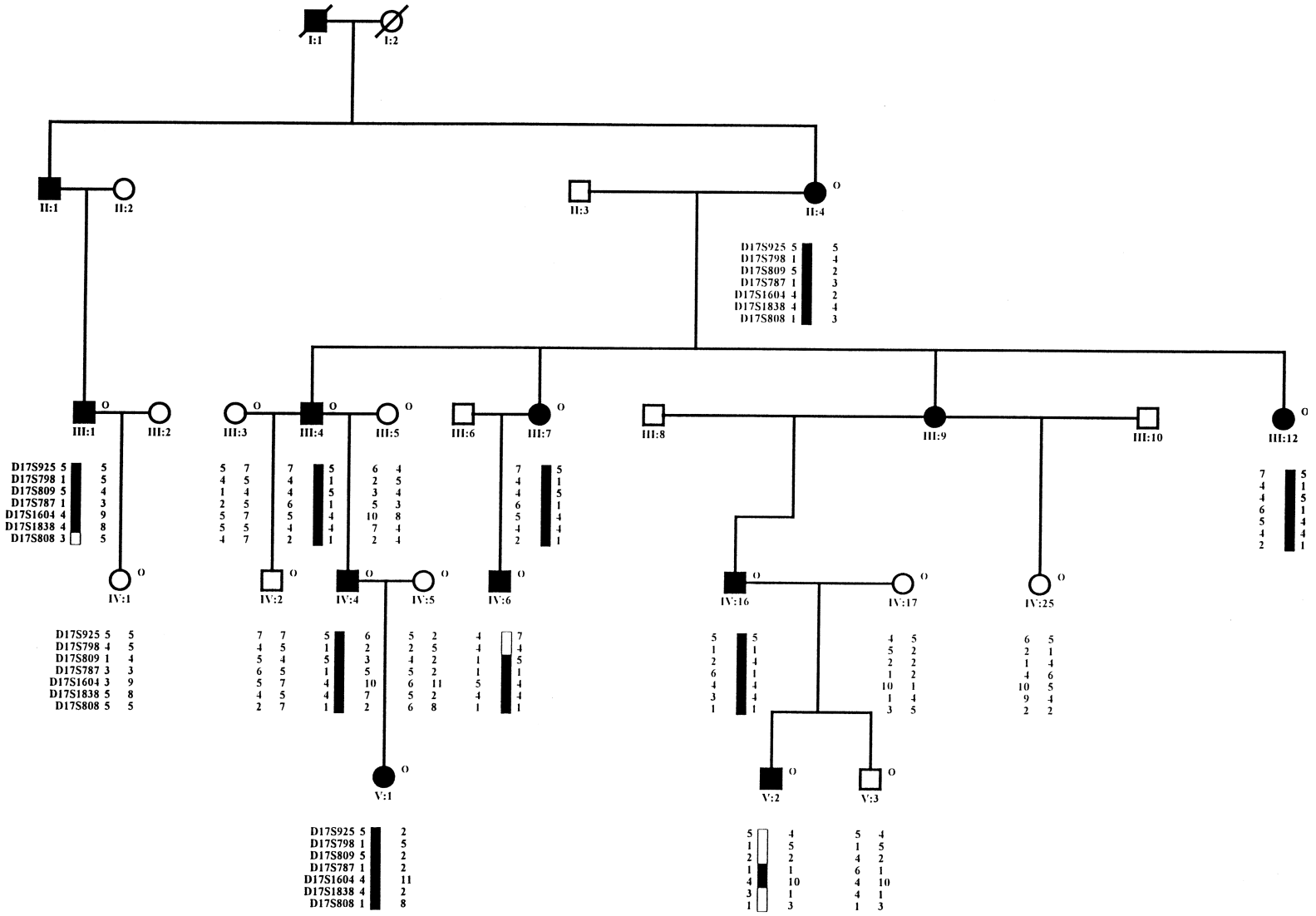

Figure 3 Haplotypes of seven DNA markers on 17q in the examined branch of the family. Unblackened circles and squares indicate members who are unaffected. Blackened circles and squares indicate members reported to be affected. A degree symbol (°) indicates tested individuals. The blackened sections of the bars indicate the haploidentical region in affected individuals, which defines the critical PTLAH region between flanking markers D17S925 and D17S808.

Table 1

Two-point LOD Scores at Seven Polymorphic Markers on Chromosome 17q

	LOD Score at θ =						
Marker	.00	.01	.03	.05	.10	.20	.30
D17S925	$-\infty$	−1.02	−.52	−.29	−.008	.19	.21
D17S798	$-\infty$	.28	.15	.31	.46	.41	.25
D17S809	$-\infty$	−.55	.28	.61	.90	.88	.61
D17S787	3.34	3.27	3.13	2.99	2.64	1.89	1.13
D17S1604	3.17	3.12	3.01	2.90	2.60	1.98	1.30
D17S1838	$-\infty$	−2.61	−1.69	−1.28	−.76	−.33	−.13
D17S808	$-\infty$	1.29	1.67	1.79	1.84	1.56	1.12

vestigations. During clinical inspection, aplastic patella was identified by the occurrence of a dimple, when legs were maintained in a closed flexed position, at the position corresponding to the anatomic site of the patella. Hypoplastic patellas were easily identified by manual palpation by their reduced sizes compared with patellas in unaffected individuals. Anatomic lesions were always bilateral. Clinical diagnosis was never questioned.

Fourteen of the 15 affected people examined complained only of minor discomfort related to the patella defect. The most severely affected subject (III-1) showed an abnormal gait, whereas walking was generally normal in other affected individuals. They complained of some difficulties with running, with stopping abruptly, with climbing the stairs, and with riding a bike. During childhood, some of them had experienced unexpected falls while walking, without additional problems. The general feeling of these persons was that they had inherited a condition that interfered only minimally with a normal lifestyle. One affected girl died at age 15 years of a cerebral aneurysm, confirmed by autopsy. No obvious internal malformations were found. On physical examination, no additional defect was found in this family, except unilateral 4th–5th syndactyly in a single subject (IV-12). In particular, no tarsal synostosis, nail dysplasia, or other dysmorphic features were present in the patients. Auxologic parameters were within normal limits, and no evidence of neurologic alterations or mental retardation was detected. Pelvic bones were normal.

Linkage Analysis

Because PTLAH is a major characteristic of a number of disorders, including NPS, aniridia, and trisomy 8q syndrome, linkage studies were first done with markers from these candidate regions. However, this analysis failed to support the location of the disease-gene on chromosome 9q, 11p, or 8q (LOD scores of $-\infty$ at recombination fraction [θ] 0 were obtained at all loci). A linkage genomewide screen was conducted with microsatellite markers at intervals of ~10 cM, and a first LOD score of 3.14 at $\theta = 0$ for the marker at locus D17S1604 was obtained. Subsequently, a LOD score of 3.34 at $\theta = 0$ was achieved for the marker at locus D17S787. Two-point LOD scores for seven additional markers from the region are given in table 1. A haplotype analysis was also done with seven markers in the interval between D17S925 and D17S808 (fig. 3). Five informative recombination events were observed in this region and allowed us to narrow down the possible locations of the disease gene. In affected individual IV-6 and in unaffected individual IV-25, a recombination event had occurred between D17S809 and D17S787, establishing the centromeric limit for the candidate region. A recombination event in the affected subjects III-1 and V-2 and in the unaffected V-3 subjects defined the telomeric boundary. Recombination mapping thus defines a 12-cM interval that must contain the PTLAH gene. This is consistent with results of multipoint analysis, which gave a maximum LOD score of 3.39, with a most likely location for the disease gene between D17S787 and D17S1604 (fig. 4).

Candidate Gene Analysis

Being closely linked to D17S787 (Gong et al. 1999), the *noggin* gene was evaluated as a first PTLAH candidate gene. A 780-bp fragment that included the *noggin*-gene single exon and part of the 5′ UTR (~400 bp) was amplified and sequenced in two affected and two unaffected family members. No mutations were found in the affected patient (fig. 5).

Discussion

We present a four-generation Venezuelan family with isolated PTLAH transmitted as an autosomal dominant mutation. We excluded linkage to the candidate chromosome regions 9q34, 11p, and 8q, where disorders associated with PTLAH have been mapped. In particular, exclusion of the NPS locus on chromosome 9q34

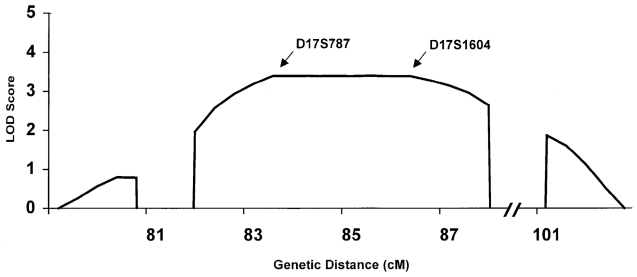

Figure 4 Multipoint LOD-score analysis for the region 17q21–22. The multipoint linkage analysis localized the locus for PTLAH within an interval of ~8 cM between markers D17S787 and D17S1604. The multipoint LOD score throughout this interval was 3.39.

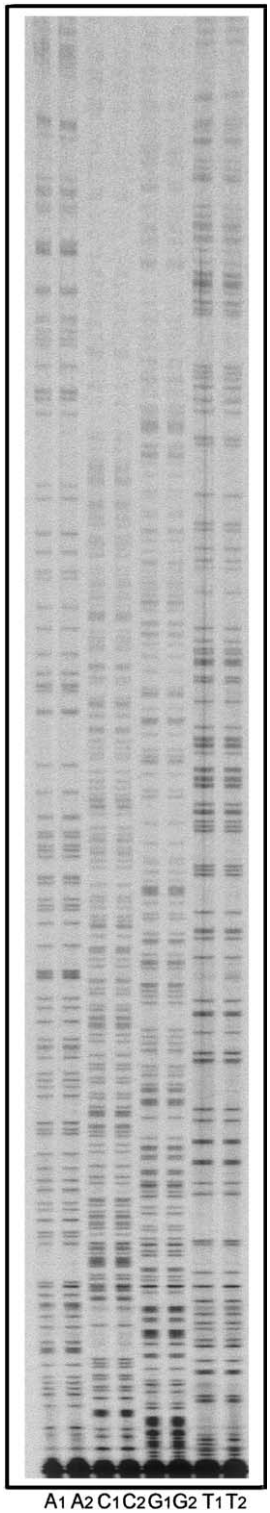

Figure 5 Comparative sequence analysis of the coding region of the *noggin* gene in patient IV:4 (*lanes A1, C1, G1, and T1*) and a normal control (*lanes A2, C2, G2, and T2*).

confirms that PTLAH is not allelic to NPS. Additional features that occur in >90% of patients with NPS—including nail dysplasia, a single iliac horn on each side, and elbow deformities—are never found in association with PTLAH (Azouz and Kozlowski 1997).

In this family, recombination studies and a multipoint analysis mapped the PTLAH gene within a 12-cM interval at 17q21–22. Genes mapped in the 17q21–22 region include the nucleolar transcription factor, cyclin A, and myeloperoxidase precursor gene, which are not likely candidates for a disorder of bone, being general-housekeeping genes (Krakow et al. 1998). Of interest, the region 17q21–22 includes DLX3 and DLX7, two members of the *distalless* gene family that encode developmental proteins expressed in neuronal and skeletal tissues (Nakamura et al. 1996). We considered the possibility that both DLX3 and DLX7 are PTLAH candidate genes. However, a recombination event in the family we studied excludes the region where these two genes are located. Thus it is unlikely that mutations in one of these two genes results in PTLAH. Of interest, Gong et al. (1999) have identified five dominant mutations within the human *noggin* gene in unrelated families segregating proximal symphalagism (SYM1 [MIM 185800]), a disease involving joint abnormalities. Because Gong et al. have positioned *noggin* between markers D17S790 and D17S794, we analyzed the complete coding sequence and part of the 5′ UTR region of this gene in patients with PTLAH, to determine whether the gene is involved in the pathogenesis of PTLAH. Sequence analysis revealed a complete absence of mutations. We are currently recruiting additional pedigrees, to narrow down the critical region and to verify genetic homogeneity of familiar PTLAH.

Acknowledgments

The authors are indebted to Dr. Ernesto Ippolito (Tor Vergata University of Rome) for useful comments on the manuscript. The Italian Ministry of Health funded this work.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Cooperative Human Linkage Center, http://www.chlc.org (for markers used for DNA typing)
Généthon, http://www.genethon.fr/
Genome Database, http://www.gdb.org (for primer sequences used for DNA typing)
Human Gene Nomenclature, http://www.gene.ucl.ac.uk/nomenclature/ (for PTLAH gene nomenclature)
Human Transcript Map, http://www.ncbi.nlm.nih.gov/science96 (for DLX3 and DLX7 genes)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for familial PTLAH [MIM 168860] and NPS [MIM 161200])

Whitehead Institute for Biomedical Research, http://www.genome.wi.mit.edu (for markers used for DNA typing)

References

Azouz EM, Kozlowski K (1997) Small patella syndrome: a bone dysplasia to recognize and differentiate from the nail-patella syndrome. Pediatr Radiol 27:432–435

Beals RK, Eckhardt AL (1969) Hereditary onycho-osteodysplasia (nail-patella syndrome). J Bone Joint Surg Am 51: 505–515

Bernhang AM, Levine SA (1973) Familial absence of the patella. J Bone Joint Surg Am 55:1088–1090

Braun HS (1978) Familial aplasia or hypoplasia of the patella. Clin Genet 13:350–352

Cassidy SB, McGee BJ, van Eys J, Nance WE, Engel E (1975) Trisomy 8 syndrome. Pediatrics 56:826–831

Curtiss J, Heilig JS (1998) Delimiting development. Bioessays 20:58–69

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Dreyer SD, Zhou G, Baldini A, Winterpacht A, Zabel B, Cole W, Johnson RL, et al (1998) Mutations in LMX1B cause abnormal skeletal patterning and renal dysplasia in nail patella syndrome. Nat Genet 19:47–50

Fineman RM, Ablow RC, Howard RO, Albright J, Breg WR (1975) Trisomy 8 mosaicism syndrome. Pediatrics 56: 762–767

Goeminne L, Dujardin L (1970) Congenital coxa vara, patella aplasia and tarsal synostosis: a new inherited syndrome. Acta Genet Med Gemellol (Roma) 19:534–545

Gong Y, Krakow D, Marcelino J, Wilkin D, Chitayat D, Babul-Hirji R, Hudgins L, et al (1999) Heterozygous mutations in the gene encoding noggin affect human joint morphogenesis. Nat Genet 21:302–304

Gorlin RJ (1990) Syndromes with unusual facies: other syndromes. In: Gorlin RJ Cohen MM Levin LS (eds) Sindromes of the head and neck, 3rd ed. Oxford University Press, New York, pp 831–832

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, et al (1994) The 1993-94 Généthon human genetic linkage map. Nat Genet 7:246–339

Jordan T, Hanson I, Zaletayev D, Hodgson S, Prosser J, Seawright A, Hastie N, et al (1992) The human PAX6 gene is mutated in two patients with aniridia. Nat Genet 1:328–332

Krakow D, Reinker K, Powell B, Cantor R, Priore MA, Garber A, Lachman RS, et al (1998) Localization of a multiple synostoses-syndrome disease gene to chromosome 17q21–22. Am J Hum Genet 63:120–124

Kutz ER (1949) Congenital absence of the patellae. J Pediatr 34:760–762

Lathrop GM, Lalouel JM (1984) Easy calculations of lod scores and genetic risks on small computers. Am J Hum Genet 36:460–465

Lucaya J, Garcia-Conesa JA, Bosch-Banyeras JM, Pons-Peradejordi G (1981) The Coffin-Siris syndrome: a report of four cases and review of the literature. Pediatr Radiol 11:35–38

Maceratesi P, Sangiuolo F, Novelli G, Ninfali P, Magnani M, Reichardt JK, Dallapiccola B (1996) Three new mutations (P183T, V150L, 528insG) and eleven sequence polymorphisms in Italian patients with galactose-1-phosphate uridyltransferase (GALT) deficiency. Hum Mutat 8:369–372

McIntosh I, Clough MV, Schäffer AA, Puffenberger EG, Horton VK, Peters K, Abbott MH, et al (1997) Fine mapping of the nail-patella syndrome locus at 9q34. Am J Hum Genet 60:133–142

McIntosh I, Dreyer SD, Clough MV, Dunston JA, Eyaid W, Roig CM, Montgomery T, et al (1998) Mutation analysis of LMX1B gene in nail-patella syndrome patients. Am J Hum Genet 63:1651–1658

Mirkinson AE, Mirkinson NK (1975) A familial syndrome of aniridia and absence of the patella. Birth Defects Orig Artic Ser 11:129–131

Nakamura S, Stock DW, Wydner KL, Bollekens JA, Takeshita K, Nagai BM, Chiba S, et al (1996) Genomic analysis of a new mammalian distal-less gene: Dlx7. Genomics 38: 314–324

O'Connell JR, Weeks DE (1995) The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set–recoding and fuzzy inheritance. Nat Genet 11:402–408

Petajan JH, Momberger GL, Aase J, Wright DG (1969) Arthrogryposis syndrome (Kuskokwim disease) in the Eskimo. JAMA 209:1481–1486

Scott JE, Taor WS (1979) The ″small patella″ syndrome. J Bone Joint Surg Br 61:172–175

Silengo MC, Davi GF, Franceschini P (1979) Radiological features in trisomy 8. Pediatr Radiol 8:116–118

Sujansky E, Smith AC, Peakman DC, McConnell TS, Baca P, Robinson A (1981) Familial pericentric inversion of chromosome 8. Am J Med Genet 10:229–235

Valenzuela DM, Economides AN, Rojas E, Lamb TM, Nunez L, Jones P, Lp NY, et al (1995) Identification of mammalian noggin and its expression in the adult nervous system. J Neurosci 15:6077–6084

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vaysseix G, et al (1992) A second-generation linkage map of the human genome. Nature 359:794–801

Wright DG (1970) The unusual skeletal findings of the Kuskokwim syndrome. Birth Defects Orig Artic Ser 6:16–24