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Genetic Heterogeneity in Multiple Epiphyseal Dysplasia

Michelle Deere,[1] Susan Halloran Blanton,[2] Charles I. Scott,[3] Leonard O. Langer,[4] Richard M. Pauli,[5] and Jacqueline T. Hecht[1]

[1]Department of Pediatrics, University of Texas Medical School, Houston; [2]Department of Pediatrics, University of Virginia, Charlottesville; [3]A. I. Dupont Institute, Wilmington, DE; [4]University of Minnesota, Minneapolis; and [5]Department of Pediatrics and Medical Genetics, University of Wisconsin, Madison

Summary

Multiple epiphyseal dysplasia (MED) comprises a group of hereditary chondrodysplasias in which there are major anatomic abnormalities of the long tubular bones. The Fairbank and Ribbing types are the most frequently cited types of MED. They are primarily defined radiographically and are autosomal dominant conditions. Recently, MED in one family was shown to map to the pericentromeric region of chromosome 19 and is probably allelic to pseudoachondroplasia. We have tested linkage with six short tandem repeat markers from chromosome 19 to autosomal dominant MED in one four-generation family and to MED in a unique family with three of seven siblings affected and with unaffected parents. Autosomal dominant MED in family 1 was linked with a maximum LOD score, at D19S212, of 3.22 at a recombination fraction (θ) of .00. Linkage to chromosome 19 was excluded with MED in the other family, under both autosomal recessive and autosomal dominant, with either reduced-penetrance or germ line–mosaicism models. Linkage to candidate genes COL9A1, COL9A2, and COL11A2 was tested and excluded for both genetic models in this family. COL11A1 was excluded under a recessive model. We have confirmed linkage of autosomal dominant Fairbank MED to chromosome 19 and have demonstrated that MED is genetically heterogeneous.

Introduction

Multiple epiphyseal dysplasia (MED) was described, by Fairbank (1947), as a chondrodysplasia in which the most marked bony abnormalities were in the epiphyses of the long tubular bones. Waddling gait, shortened extremities, genu valgum, and early-onset degenerative joint disease are common clinical findings. Two clinical phenotypes, Fairbank and Ribbing, have been described (Spranger et al. 1974). However, in some individuals it is not possible to differentiate the specific type of MED. It has been suggested that these two types may represent variability within the same disorder (Kozlowski and Lipska 1967). Both types are predominantly transmitted in an autosomal dominant pattern, but families have been reported with apparent autosomal recessive inheritance (Ribbing 1937; Waugh 1952; Hunt et al. 1967; Juberg and Holt 1968; Gamboa and Lisker 1974). Recently, autosomal dominant MED in one family has been mapped to chromosome 19, EDM1 (Oehlmann et al. 1994), and in another family to chromosome 1, EDM2 (Briggs et al. 1994). In the present study, we have tested linkage between chromosome 19 markers and MED in two additional families. One family has autosomal dominant Fairbank MED, and one family has three of seven affected siblings and unaffected parents.

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Address for correspondence and reprints: Dr. Jacqueline T. Hecht, University of Texas Medical School at Houston, P.O. Box 20708, Houston, TX 77225.

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Subjects and Methods

Two families with MED were ascertained through the genetic clinics at the University of Texas Medical School at Houston and the University of Wisconsin at Madison (fig. 1). Family 1 demonstrated an autosomal dominant pattern of inheritance, while family 2 had three of seven siblings affected with MED and had normal parents. Clinical and radiographic information will be presented on both families.

Family 1

The proband, a 5-year-old Hispanic female (fig. 1, IV-9), her 8-year-old sister (fig. 1, IV-10), and her 12-year-old brother (fig. 1, IV-7) were presented for genetic evaluation because of painful hips and waddling gait. They all had stocky body habitus, but height was within ±2 SD and ranged from the 35th to the 70th percentile. Head circumference and weights were within ±2 SD. None were disproportionate, since the arm spans approximated the heights. The hands were not short, and measurements ranged from the 10th to the 75th percentile for age.

Radiographic findings on all three subjects showed typical features of the Fairbank type of MED. All the epiphyses were small, irregular, and flat, especially at the knees (fig. 2). The femoral necks were short and broad, and the capital

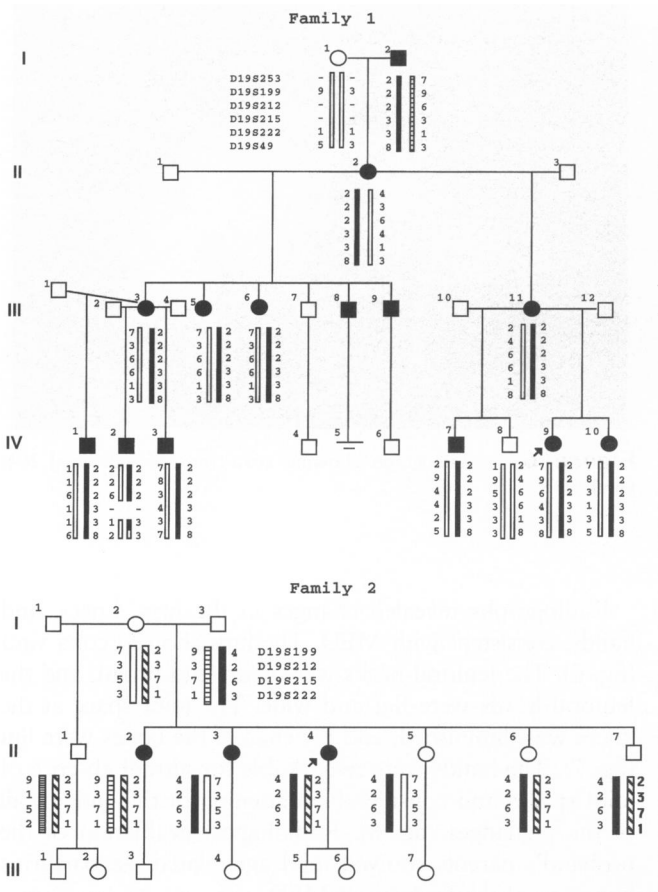

Figure 1 Chromosome 19 haplotypes of MED families

femoral epiphyses were small and round (fig. 3). The bones of the hand were normal, with delayed ossification of the distal ulnar epiphyses and carpal bones (fig. 4).

A family history of a four-generation vertical pattern of inherited hip abnormalities was consistent with autosomal dominant inheritance (fig. 1). The patient's mother had bilateral hip and knee replacements at 20 and 30 years of age, respectively. All affected adults had bilateral total hip replacements in early adulthood. All affected individuals

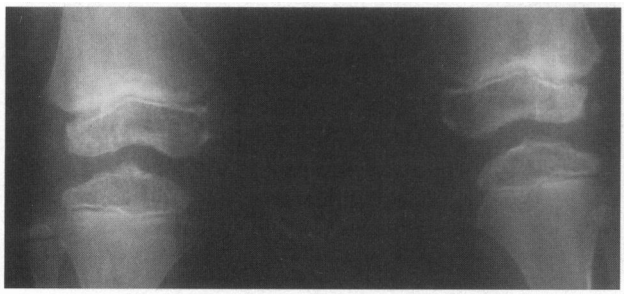

Figure 2 Radiograph of individual IV-9, family 1, at 5 years of age, showing flat epiphyseal centers at the knees.

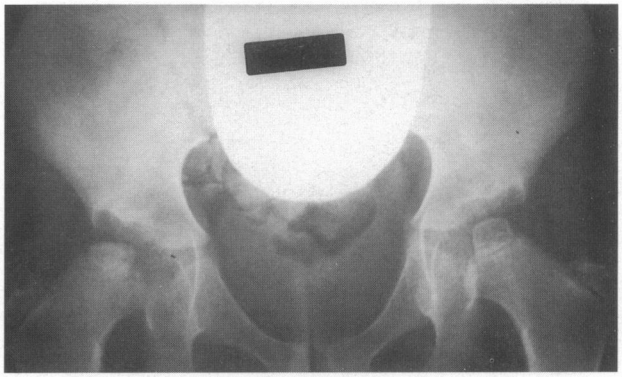

Figure 3 Capital femoral epiphyses of individual IV-9, family 1, at 5 years of age, which are small, irregular, and flat.

had heights within ±2 SD. Radiographic findings in the patient's mother were typical of MED. Other family members had been treated at orthopedic facilities and had diagnoses of MED.

Family 2

The proband, a 43-year-old white female (fig. 1, II-4), was evaluated because of short stature and joint pain. She

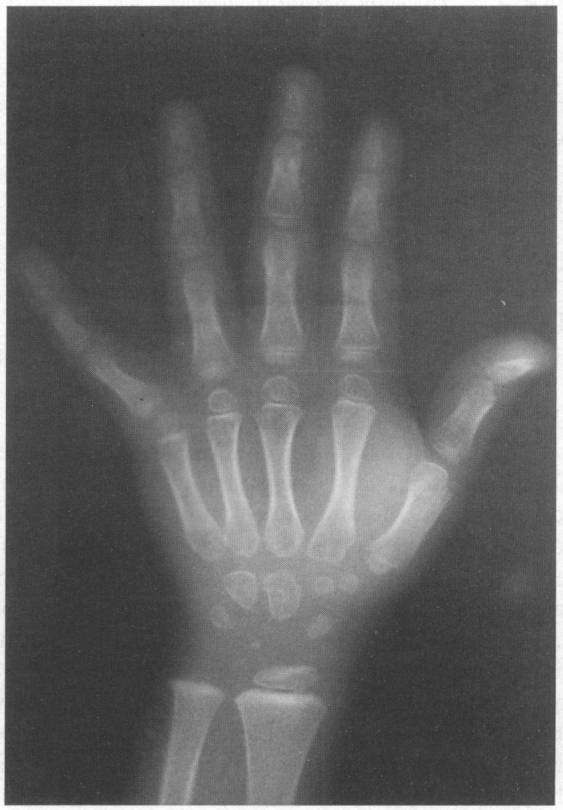

Figure 4 Hand radiograph of individual IV-9, family 1, at 5 years of age, showing delayed ossification.

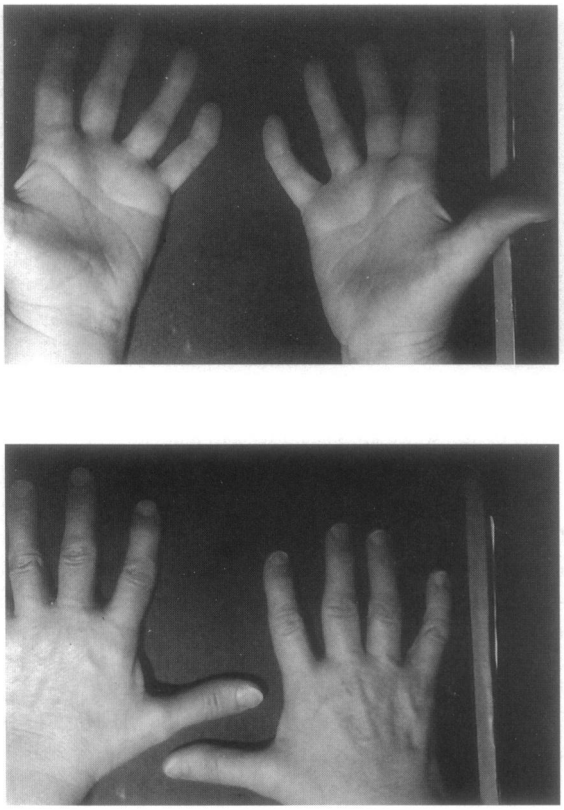

Figure 5 Prominent metacarpal/phalangeal and interphalangeal joints in individual II-4, family 2.

complained of joint stiffness, periodic midback pain, and mild pain in all of her joints, beginning in early adulthood. Climbing stairs and arising from kneeling and sitting positions were difficult.

Family history revealed two other siblings (fig. 1, II-2 and II-3) who also had joint pain and short stature (fig. 1). The parents were of average stature, 185 and 163 cm, and neither complained of joint pain. The other three full siblings and one maternal half-sibling were of average stature and had no joint-related complaints.

On physical exam, the patient's height was 150.1 cm (< -2 SD), arm span 143.5 cm, and head circumference 55.7 cm (within ± 2 SD). The patient was generally eumorphic, and physical findings were primarily related to joint involvement. Arms showed mild proportionate shortening. The hands showed markedly prominent metacarpal/phalangeal and interphalangeal joints that had limited mobility (fig. 5). Hand measurements were at the 25th percentile. A right concave scoliosis was present in the lower thoracic spine. Hip mobility was normal. The left knee showed marked lateral instability. Ankles had limited dorsiflexion and showed puffy prominences inferior of the lateral malleoli bilaterally. Results of a neurological exam were normal, except for minimal weakness in the legs, which was thought to be secondary to disuse.

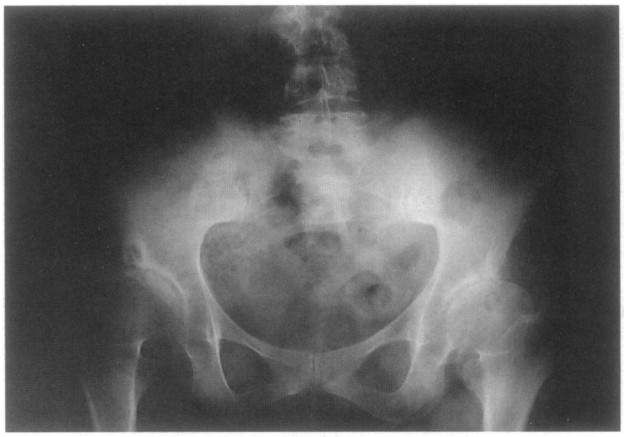

Figure 6 Radiograph showing coxa vara of individual II-4, family 2.

Radiographs revealed changes in the hips, knees, and hands, consistent with MED. The hips showed coxa vara (fig. 6). The femoral necks were short and broad, and the femoral heads were flat and wide. The joint space at the knees was diminished, and the ends of the bones were flat (fig. 7). The hands were remarkable for virtual absence of joint spaces and considerable flattening of the ends of all of the phalanges (fig. 8). Radiological evaluation of the proband's parents showed mild age-related osteoarthritis but none of the findings of MED.

Brief physical assessment of one of the proband's affected sisters showed virtually identical findings, while her mother showed none of the unusual features. The proband's unaffected siblings showed no evidence of MED. Previous pediatric and orthopedic assessments support the assumption that the three sisters showed physical and radiological features discontinuous with those of other family members.

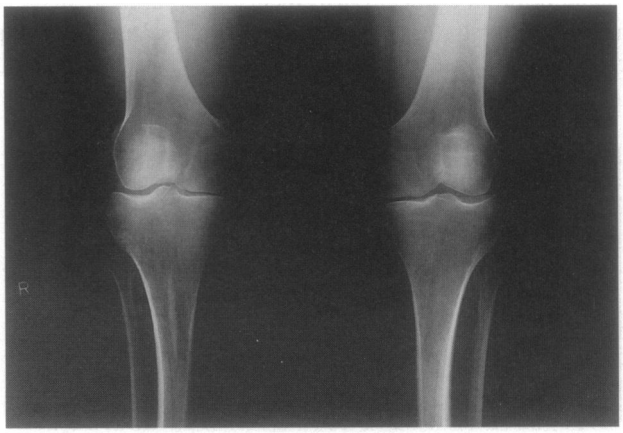

Figure 7 Radiograph of individual II-4, family 2, showing flat and wide femoral necks and flat femoral heads.

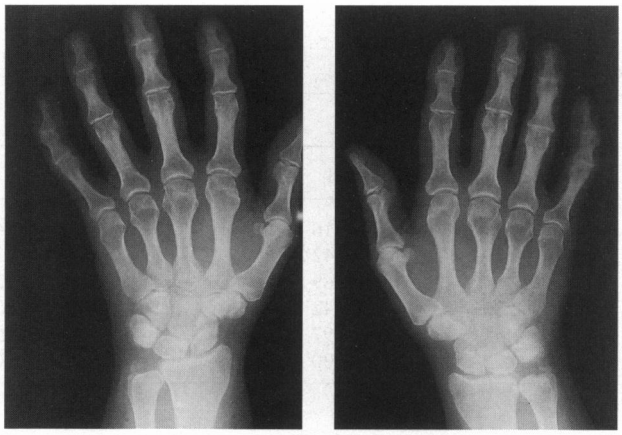

Figure 8 Hand radiographs of individual II-4, family 2, showing absence of the joint spaces and flattening of all the ends of the phalanges.

Methods

Blood samples were collected from members of the two families, and DNA was extracted from white blood cells by phenol-chloroform extractions following established protocols (Sambrook et al. 1989). Linkage to six chromosome 19 short tandem repeat PCR markers—D19S253, D19S199, D19S212, D19S215, D19S222, and D19S49—was tested. Primer and mapping information has been published elsewhere (Weber et al. 1993). Linkage was tested in family 2 to short tandem repeat markers in candidate genes COL9A1 (8B211 and 12B111) and COL11A1 and to linked markers D6S105, D6S276, D6S273, and D6S291 for COL11A2 and LMYC, D1S211, D1S197, and D1S193 for COL9A2.

DNA was amplified in 10-μl PCR reactions containing 0.1 mg of DNA and 1 mM of each primer. Samples were amplified for 30 cycles in a Cetus 480 thermal cycler with denaturation at 94°C for 45 s, annealing that varied, depending on the marker, from 45°C to 60°C for 30 s, and extension at 72°C for 30 s, with a final 10-min extension at 72°C. Samples were analyzed on 6% denaturing polyacrylamide gels. The gels were visualized using the Gelcode silver-staining kit.

Two-point LOD scores were generated using MLINK of the LINKAGE package (version 5.03) (Lathrop et al. 1984). MED was assumed to be an autosomal dominant disorder with 98% penetrance in the heterozygote and 100% penetrance in the homozygote. To allow for germline mosaicism in family 2, all unaffected individuals were considered to be unknown for MED status. A frequency

Table 1

LOD Scores for All MED Families and Chromosome 19 Markers

	LOD SCORE AT θ =						
LOCUS AND FAMILY	.00	.001	.01	.05	.10	.20	.30
D19S253:							
1	1.73	1.73	1.70	1.60	1.47	1.18	.85
2	.18	.18	.17	.15	.12	.07	.03
Total	1.91	1.91	1.87	1.75	1.59	1.25	.88
D19S199:							
1	2.47	2.46	2.42	2.22	1.97	1.45	.90
2	−2.47	−2.13	−1.37	−.72	−.44	−.19	−.08
Total	.00	.33	1.05	1.50	1.53	1.26	.82
D19S212:							
1	3.22	3.21	3.17	2.98	2.72	2.17	1.54
2	−.30	−.30	−.28	−.22	−.17	−.09	−.04
Total	2.92	2.91	2.89	2.76	2.55	2.08	1.50
D19S215:							
1	2.90	2.90	2.86	2.68	2.46	1.96	1.40
2	−2.47	−2.13	−1.37	−.72	−.44	−.19	−.08
Total	.43	.77	1.49	1.96	2.02	1.77	1.32
D19S222:							
1	1.86	1.86	1.83	1.69	1.50	1.11	.68
2	−2.47	−2.13	−1.37	−.72	−.44	−.19	−.08
Total	−.61	−.27	.46	.97	1.06	.92	.60
D19S49:							
1	−2.05	−1.79	−1.10	−.50	−.28	−.11	−.04
2	.00	.00	.00	.00	.00	.00	.00
Total	−2.05	−1.79	−1.10	−.50	−.28	−.11	−.04

Table 2

LOD Scores for Family 2 and Chromosome 19 Markers, under an Autosomal Recessive Model.

	LOD SCORE AT θ =						
MARKER	.00	.001	.01	.05	.10	.20	.30
D19S253	−.22	−.22	−.22	−.20	−.16	−.10	−.04
D19S199	−∞	−7.42	−4.41	−2.30	−1.42	−.61	−.23
D19S212	−∞	−2.92	−1.89	−1.08	−.69	−.31	−.12
D19S215	−∞	−7.42	−4.41	−2.30	−1.42	−.61	−.23
D19S222	−∞	−5.02	−3.01	−1.58	−.97	−.41	−.15
D19S49	.00	.00	.00	.00	.00	.00	.00

of .00002 was assumed for the MED allele, and a new mutation rate of .0004 was assumed for both males and females. Little or no information is available on the prevalence or mutation rate of MED. For this reason, we arbitrarily used a prevalence based on the frequency of achondroplasia, as well as an achondroplasia mutation rate, which, on the basis of clinical experience, are likely overestimates for MED. Family 2 was also tested for markers to chromosome 19, under an autosomal recessive model, and to other candidate loci, under both autosomal dominant and recessive models. For the autosomal recessive model, an allelic frequency of .00446 was assumed for MED, and the disorder was assumed to be fully penetrant in the homozygous state.

Results

Results of the two-point analyses between MED and markers on chromosome 19 are presented in table 1. When both families were considered under the autosomal dominant model, the maximum LOD score observed was 2.92 at a recombination fraction (θ) of .00 for D19S212. However, a maximum LOD score of 3.22 at θ = .00 for D19S212 was obtained for family 1 (table 1). Multipoint

Table 3

LOD Scores for Family 2 and Candidate Genes

	LOD SCORE AT θ =						
MARKER	.00	.001	.01	.05	.10	.20	.30
Dominant model:							
COL9A1	−1.77	−1.77	−1.76	−1.58	−1.17	−.54	−.21
LMYC	−1.60	−1.60	−1.56	−1.15	−.75	−.32	−.12
D1S211	−2.47	−2.13	−1.37	−.72	−.44	−.19	−.08
D1S193	−2.47	−2.13	−1.37	−.72	−.44	−.19	−.08
D1S197	−.30	−.30	−.28	−.22	−.17	−.09	−.04
COL11A1	−.30	−.30	−.28	−.22	−.17	−.09	−.04
D6S105	−1.59	−1.59	−1.50	−1.07	−.07	−.30	−.11
D6S276	.11	.11	.11	.09	.07	.04	.02
D6S273	−2.47	−2.13	−1.37	−.72	−.44	−.19	−.08
D6S291	−.30	−.30	−.28	−.22	−.17	−.09	−.04
Recessive model:							
COL9A1	−2.47	−2.13	−1.37	−.72	−.44	−.19	−.08
LMYC	−2.47	−2.13	−1.37	−.72	−.44	−.19	−.08
D1S211	−∞	−7.42	−4.41	−2.30	−1.42	−.61	−.23
D1S193	−∞	−7.42	−4.41	−2.30	−1.42	−.61	−.23
D1S197	−∞	−2.92	−1.89	−1.08	−.69	−.31	−.12
COL11A1	−∞	−2.32	−1.30	−.65	−.39	−.16	−.06
D6S105	−2.47	−2.13	−1.37	−.72	−.44	−.19	−.08
D6S276	−∞	−2.61	−1.56	−.72	−.37	−.09	−.01
D6S273	−∞	−7.42	−4.41	−2.30	−1.42	−.61	−.23
D6S291	−∞	−2.92	−1.89	−1.08	−.69	−.31	−.12

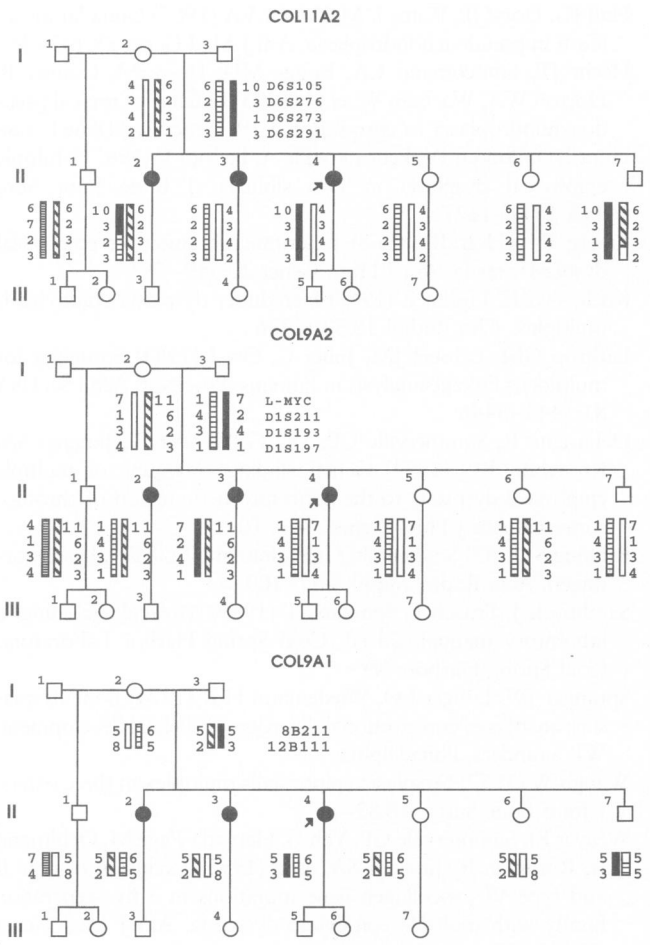

Figure 9 Family 2 haplotypes

analysis on family 1 suggested a placement of EDM1 telomeric to D19S49 (data not shown). However, a previous study localized the gene between D19S212 and D19S215 (Oehlmann et al. 1994). As can be seen in tables 1 and 2, under both the autosomal dominant model and the recessive model, family 2 is also excluded from this region of chromosome 19. Haplotype analysis shows that the affected individuals, II-2 and II-3, inherited different parental chromosomes (fig. 1).

Linkage to the candidate loci was also tested under both autosomal dominant and recessive models, in family 2 (table 3 and fig. 9). Under the autosomal dominant model, COL9A1, D6S105, D6S273, and LMYC were excluded. D6S276 and COL11A1 were not informative under this model. Haplotype analysis of chromosome 6 markers showed discordant inheritance of parental haplotypes, thus excluding COL11A2 in this family (fig. 9). Linkage to all of the candidate genes and linked markers was excluded under the autosomal recessive model.

Discussion

Linkage of MED to chromosome 19 was based on the findings in a single family (Oehlmann et al. 1994). The clinical and radiographic findings described in this family are typical of the Fairbank type of MED (Weaver et al. 1993). MED shows variability including two clinical types, suggesting that there may be genetic heterogeneity. We evaluated two additional families with MED, for linkage to chromosome 19. Autosomal dominant Fairbank type MED in one of the families showed linkage to chromosome 19. A significant LOD score was found for D19S212, confirming the previous linkage findings. Only one recombinational event occurred in family 1, between D19S222 and D19S49, strengthening the conclusion that EDM1 lies above D19S222 (fig. 1) (Oehlmann et al. 1994).

We also tested family 2, assuming that MED is transmitted either as an autosomal dominant disease with decreased penetrance, or as germ-line mosaicism, or as an autosomal recessive disorder. Linkage, under either model, was excluded. Haplotype analysis revealed that affected individuals II-2 and II-3 did not share a common haplotype for this region of chromosome 19 (fig. 1). Furthermore, patient II-4 shared an identical haplotype with two of her unaffected siblings, II-6 and II-7, as did patient II-3 with unaffected sibling II-5. These results indicate that MED is a genetically heterogeneous disease.

The physical and radiographic findings on the proband in family 2 are consistent with a diagnosis of MED, but neither of the parents has any of the expected symptoms of MED. Therefore the etiology of the disease is not the typical autosomal dominant form. In the literature there are reports of cases of MED with autosomal recessive inheritance (Ribbing 1937; Waugh 1952; Hunt et al. 1967; Juberg and Holt 1968; Gamboa and Lisker 1974), but in none of the families can autosomal dominant inheritance with germ-line mosaicism be ruled out. Although germ-line mosaicism has not been reported in MED, it has been demonstrated in pseudoachondroplasia, a more severe dwarfing condition that has also been mapped to chromosome 19 (Hall et al. 1987; Hecht et al. 1993). Since MED and pseudoachondroplasia may be allelic conditions, germline mosaicism may not be unexpected. However, the haplotypes of the three affected sisters in this family exclude germ-line mosaicism at the chromosome 19 locus. All three of the affected individuals in this MED family have reproduced. None of the children complain of joint pain at 21, 23, 25, and 26 years of age, but none have been assessed formally; and the lack of MED findings could be due to the late onset of this disease.

Further, we have tested and excluded cartilage-specific candidate genes—COL9A1, COL9A2, and COL11A2—under both autosomal dominant and recessive models in family 2. COL11A1 has been excluded under an autosomal

recessive model. COL11A2 maps between D6S273 and D6S291 (G. E. Tiller, personal communication). The crossover in family 2 (individual II-2) is between D6S276 and D6S273. However, none of the affected individuals have the same haplotype. COL9A2 has recently been reported to be linked in one family with autosomal dominant MED (Briggs et al. 1994). This suggests that at least three loci may be involved in causing MED in different families.

In summary, we have confirmed that autosomal dominant Fairbank MED maps to chromosome 19 and have shown that MED in one family does not map to chromosome 19. In addition, MED in this latter family does not show linkage to the recently reported chromosome 1 location of MED. Thus, at least one additional genetic locus remains to be identified for conditions having the clinical and radiographic criteria of MED.

Acknowledgments

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