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Acromesomelic Dysplasia Maroteaux Type Maps to Human Chromosome 9

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Summary

Acromesomelic dysplasias are skeletal disorders that disproportionately affect the middle and distal segments of the appendicular skeleton. We report genetic mapping studies in four families with acromesomelic dysplasia Maroteaux type (AMDM), an autosomal recessive osteochondrodysplasia. A peak LOD score of 5.1 at recombination fraction 0 was obtained with fully informative markers on human chromosome 9. In three of the four families, the affected offspring are products of consanguineous marriages; if it is assumed that these affected offspring are homozygous by descent for the region containing the AMDM locus, a 6.9-cM AMDM candidate interval can be defined by markers D9S1853 and D9S1874. The mapping of the AMDM locus to human chromosome 9 indicates that AMDM is genetically distinct from the two other mapped acromesomelic dysplasias, Hunter-Thompson type and Grebe type, which are caused by mutations in CDMP1 on human chromosome 20.

Introduction

Heritable osteochondrodysplasias comprise a diverse group of disorders in which the sizes and/or shapes of skeletal elements are abnormal (Rimoin et al., in press). In many cases, linkage mapping of these disorders, followed either by positional-candidate analysis or positional cloning, has led to the identification of genes and pathways important to the process of skeletal growth (reviewed in Mundlos and Olsen 1997*a*, 1997*b*). Grouping phenotypically distinct disorders on the basis of shared pathological or radiological features (Spranger 1985) has facilitated this molecular genetic dissection. For example, that allelic mutations would cause three clinically distinct disorders—achondroplasia, hypochondroplasia, and thanatophoric dysplasia—was suspected on the basis of their radiological similarities (Mundlos and Olsen 1997*a*). Conversely, disorders having dissimilar radiological or pathological features, despite clinical similarities, are likely to be etiologically different. This is exemplified in the acromesomelic dysplasias, disorders in which there is disproportionate shortening of skeletal elements, predominantly affecting the middle segments (forearms and forelegs) and distal segments (hands and feet) of the appendicular skeleton.

A number of phenotypically distinct heritable skeletal disorders with acromesomelic shortening have been described (Maroteaux et al. 1971; Beighton 1974; Hunter and Thompson 1976; Cantú et al. 1977; Osebold et al. 1985; Brahimi et al. 1988; Sener et al. 1993; Pfeiffer et al. 1995; Ferraz et al. 1997). Among those with autosomal recessive inheritance, the specific name "acromesomelic dwarfism" was used by Maroteaux et al. (1971) to describe a distinct phenotype in three patients, two of whom were siblings. Hunter and Thompson (1976) described a patient whose involvement also followed an acromesomelic pattern and noted that their patient's features were different from those reported by Maroteaux et al. (1971) but clinically similar to two sisters reported by Grebe (1952). Langer et al. (1989) concluded that the acromesomelic dysplasia described by Hunter and Thompson (AMDH) and that reported by Grebe (AMDG) are radiologically related but not identical; this relatedness has been confirmed at the molecular genetic level, as both disorders are caused by allelic mutations in the cartilage-derived morphogenetic

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protein-1 gene, CDMP1, on human chromosome 20 (Thomas et al. 1996, 1997).

Individuals with either AMDH or AMDG have normal axial skeletons and missing or fused skeletal elements within their hands and feet. This contrasts with the radiological features of acromesomelic dysplasia Maroteaux type (AMDM [MIM 201250]). In patients with AMDM, all skeletal elements are present, but they have abnormal rates of linear growth (Langer and Garrett 1980). In addition, axial skeletal involvement occurs in individuals with AMDM, characterized by wedging of vertebral bodies, with the dorsal margins being shorter than the ventral margins (Langer and Garrett 1980). These clinical differences, which serve to distinguish AMDM from AMDH and AMDG, suggest that AMDM is also genetically distinct. Herein, we describe in detail one family (fig. 1) affected with AMDM, and we report the genetic mapping of the disorder, using DNA from four families, to human chromosome 9p13-q12.

Subjects, Material, and Methods

Clinical Description

The index patient (family 1, individual IV:5; fig. 2), a 34-year-old woman, has a height of 125 cm, a high forehead, a normal head circumference, and nondysmorphic facies. Her limbs are disproportionately short. Her fingers are extremely short and broad, with redundant skin (fig. 3). A skeletal survey reveals a normal skull and short tubular bones of the arms and legs, with broad metaphyses (fig. 4). There is no disproportionate mesomelic shortening of the arms and no dislocation of the radial head. In the hands, the phalanges and metacarpals are short and broad (fig. 4). In the feet, the phalanges and metatarsals are also short and broad, with disproportionately larger bones of the great toe in comparison with the other toes. There is a narrow spinal canal in the thoraco-lumbar region, due to a narrow interpedicular distance (Th12-L4) and bulging of disci. The interpedicular distance in the sacral region is normal. All lumbar vertebrae are wedge shaped, with the dorsal margins shorter than the ventral margins.

Three other siblings (IV:1, IV:7, and IV:10) have the same condition. The youngest affected sister (IV:10), who is 25 years old and 122 cm tall, also has bowing of the forearms, with limited extension of the elbows. X-rays of her left arm and hand show shortening of all tubular bones, with a disproportionate shortening of the radius and ulna, in comparison with the humerus (fig. 5). The radial head is luxated. An X-ray of her spine, at age 16 years, shows anterior hypoplasia of the first lumbar vertebral body, whereas the second to fifth lumbar vertebral bodies are wedge shaped (fig. 5). Seven other siblings, of whom one died at age 9 years from a congenital heart malformation, have normal height, and all siblings have normal intelligence. The parents of these siblings are first cousins.

Figure 1 Three affected siblings with acromesomelic dysplasia (*foreground*), their mother (*left*), and five of their unaffected siblings.

Three other families with clinical and radiological features of AMDM were identified. Clinical features common to all four families included short stature with disproportionate acromelic shortening and variable mesomelic shortening of the limbs, normal intelligence, nondysmorphic facies, and the absence of other organ-system involvement. Radiologic features indicative of AMDM (Langer and Garrett 1980) were present in all affected individuals. These features included short and broad phalanges and metacarpals in the hands; short tubular bones of the arms and legs, with broad metaphyses; and wedge-shaped lumbar vertebrae, with dorsal margins shorter than ventral margins. Unaffected parents in all families and parental consanguinity in three of the four families (families 1, 2, and 4; fig. 2) support autosomal recessive inheritance.

DNA Collection and Genotyping

Informed consent was obtained from all participants, and blood was collected for DNA extraction. All polymorphic markers used for linkage analysis were simple-sequence–repeat polymorphisms (SSRPs) purchased from Research Genetics. Markers were PCR amplified in 20-μl reactions containing 1 × PCR buffer, 50 ng genomic DNA, 200 μM each dNTP, 2 pmol each primer, and 0.1 U *Taq* polymerase. All forward primers were

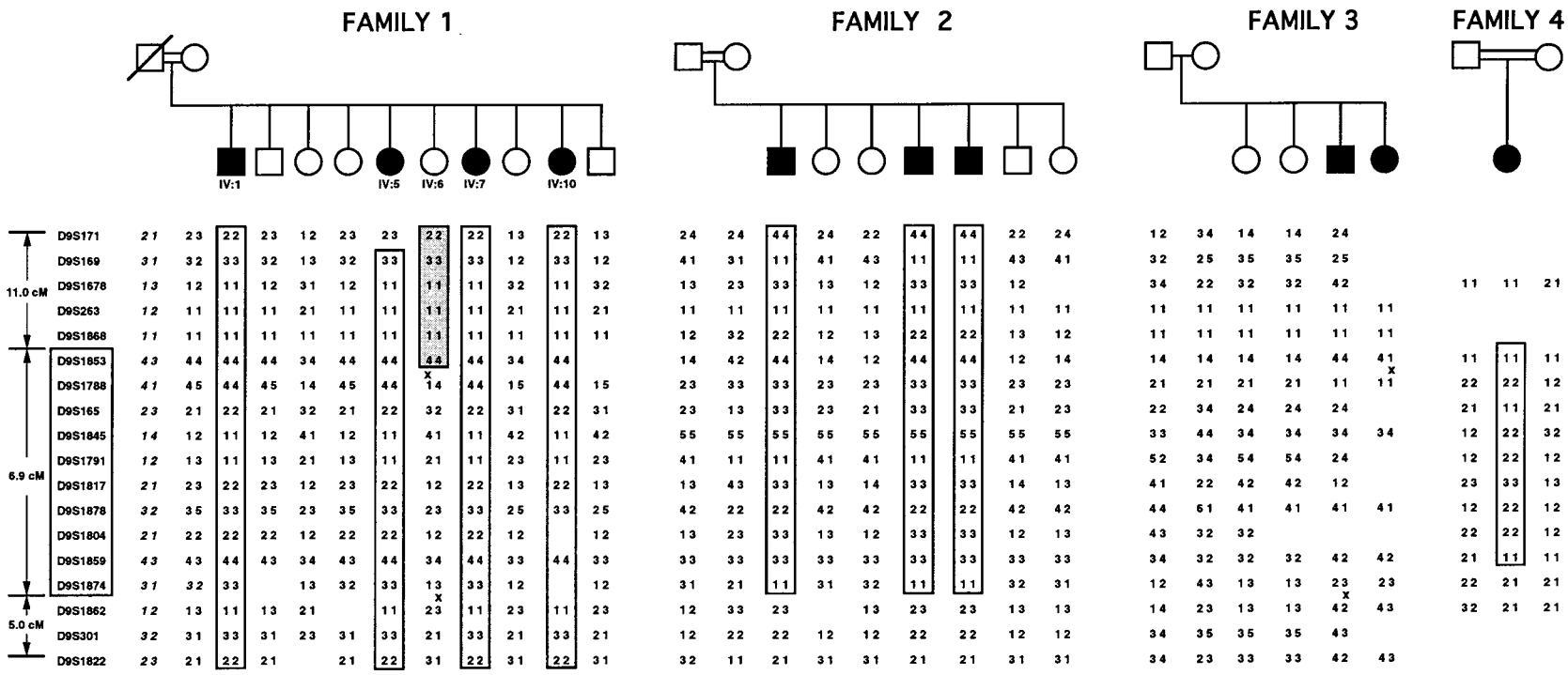

Figure 2 Pedigrees and genotype data for markers spanning the AMDM candidate interval. Markers are shown in order, p telomeric to q telomeric, and are based on published linkage maps. The boxed markers define the AMDM candidate region. Genetic distances between key markers are indicated. A deduced genotype of the deceased father in family 1 is provided in italics. For offspring, paternally derived alleles are on the left and maternally derived alleles are on the right. Incomplete genotypes are blank. Sites of likely meiotic recombination are marked with an "x." Boxed genotypes indicate markers compatible with homozygosity by descent in affected offspring. The shaded boxed genotype in family 1 depicts the region of shared identity by descent between an unaffected sibling and her affected siblings. Parents in families 1, 2, and 4 are consanguineous; parents in family 3 are unrelated. Families are unrelated to each other.

end-labeled with ^{33}P by use of T4 polynucleotide kinase. PCR conditions included an initial denaturation of 4 min at 95°C, followed by 35 cycles of 40 s at 95°C, 40 s at 56°C, 60 s at 72°C, and a final extension of 10 min at 72°C. PCR products were separated by denaturing-gel electrophoresis, and alleles were detected by autoradiography.

Linkage Calculations

The MLINK subroutine of the LINKAGE software package (Lathrop et al. 1984) was used to calculate two-point LOD scores for fully informative markers contained within the candidate region. Because ethnically and geographically matched control-allele frequencies were not available for each of the three consanguineous kindreds, each of whom is from a different ethnic group and a different geographic region, linkage calculations did not include inbreeding loops. All calculations assumed autosomal recessive inheritance with complete penetrance, a mutant allele frequency of 10^{-2} in the control population, and a phenocopy frequency of 10^{-5}.

Results

When the mapping of AMDM was initiated, DNA was available only from family 1. Linkage to CDMP1 was excluded in this family by use of an intragenic SSRP (data not shown). However, even with four affected children and six unaffected children, family 1 would not be large enough to yield a significant LOD score (LOD >3 at recombination fraction [θ] 0) by standard linkage analysis. Since the children in this family had consanguineous parents, however, the inclusion of inbreeding loops in the linkage calculations could lead to the detection of significant linkage with a marker allele whose frequency in the general population is low (Lander and Botstein 1987). Consequently, a DNA-pooling approach was used both to identify informative markers, which could be used for standard linkage calculations, and to identify markers suggestive for homozygosity by descent in the affected members of the family. Markers from the Research Genetics screening set (version 6A), which contains SSRPs spaced an average of 25 cM apart, were tested on two pooled DNA samples. One pool contained equal quantities of DNA from the four affected siblings; the other pool contained equal quantities of DNA from four of the unaffected siblings (DNA from two additional unaffected siblings was not available at the time that the pooling experiments were performed). We used 50 ng of each pooled sample as template for PCR.

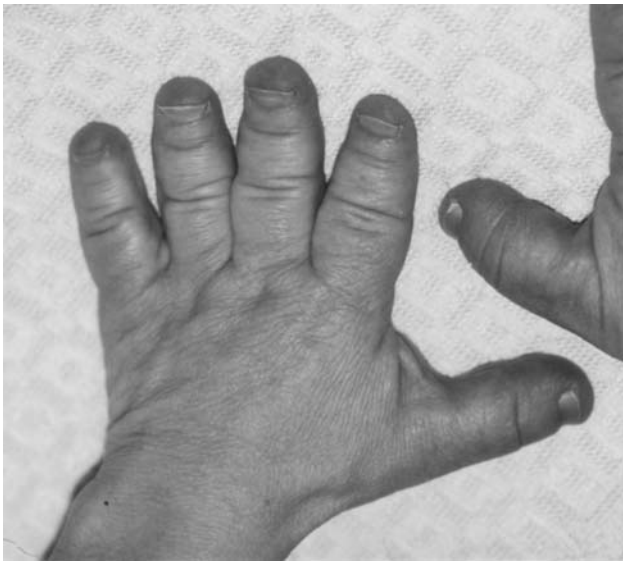

Figure 3 Hand of individual IV:5. Note short, broad fingers with redundant skin.

Under the assumption that the affected individuals from this kindred are homozygous by descent for their acromesomelic dysplasia mutation, a fully informative marker tightly linked to the disease locus would yield only a single allele in the affected-sibling pooled sample and two or three alleles in the unaffected-sibling pooled sample. Uninformative markers would yield only a single allele in both the affected- and the unaffected-sibling pooled samples, whereas partially informative markers would yield one or two alleles.

Ninety-six markers from chromosomes 1–11 were tested on the two pooled samples. Two of these 96 markers, D2S1326 and D9S301, yielded a single allele in the affected-sibling pooled sample and three alleles in the unaffected-sibling pooled sample. These two markers and their nearby flanking markers (determined from Dib et al. 1996) were then tested on the individual DNA samples in this family. Only those markers on chromosome 9, in the region of D9S301, yielded results compatible with homozygosity by descent. All affected members in family 1 were homozygous for a contiguous series of 17 markers spanning >15 cM, from D9S169 to D9S1822 (fig. 2).

DNA from the three other families was then collected and tested with the chromosome 9 markers. Results in family 2 were also consistent with homozygosity by descent for markers in this region. Affected members of this family were homozygous for a contiguous series of 15 markers spanning >20 cM (fig. 2). Nine contiguous markers from this region were also homozygous in the affected individual from family 4 (fig. 2). In family 3, which was nonconsanguineous, results were also consistent with linkage to this region (fig. 2).

Analysis of genotypes in the four families permits the determination of the AMDM candidate region's boundaries. At present, the p arm's telomeric boundary is defined by D9S1853. An unaffected individual (IV:6) in

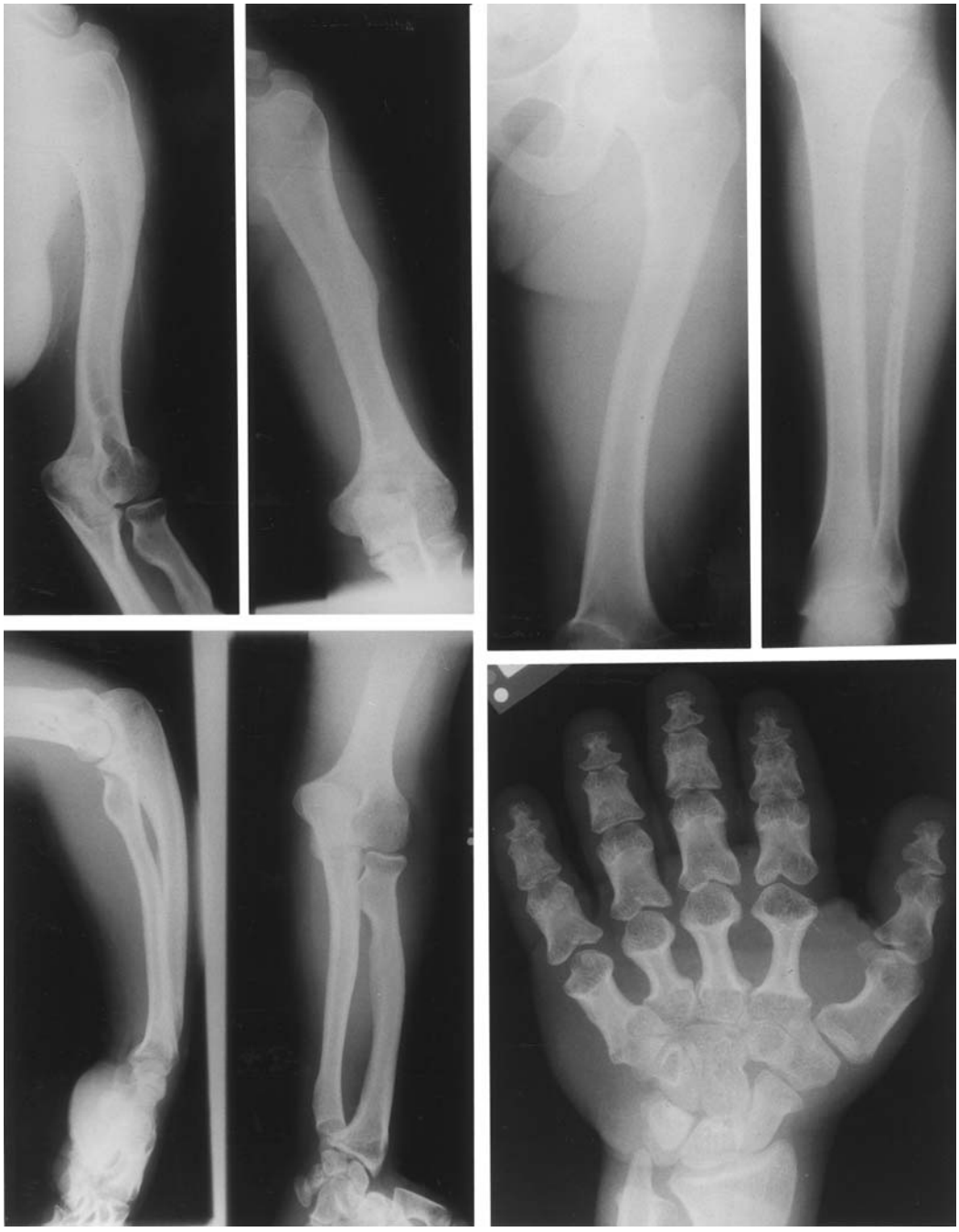

Figure 4 X-rays for individual IV:5 (clockwise from top left): humerus (two views), femur, tibia and fibula, hand, radius, and ulna (two views). All tubular bones are short. Widening of the metaphysis is apparent in the distal humerus (*top left*). Phalanges and metacarpals in the hands are short and broad; all carpal, metacarpal, and phalangeal bones are present, and none are abnormally fused (*bottom right*).

family 1 is identical by descent for this marker, and those immediately telomeric of it, with her affected siblings. If complete penetrance for the phenotype is assumed, markers for which this unaffected individual is identical by descent with her affected siblings must be excluded from the candidate region (shaded box in fig. 2). Further supporting D9S1853 as the p-arm telomeric boundary is a maternal meiotic recombination event in family 3, which makes the two affected siblings in this family discordant for this marker and those p telomeric of it. The q-arm telomeric boundary of the candidate interval is defined by marker D9S1874 in family 4. This marker,

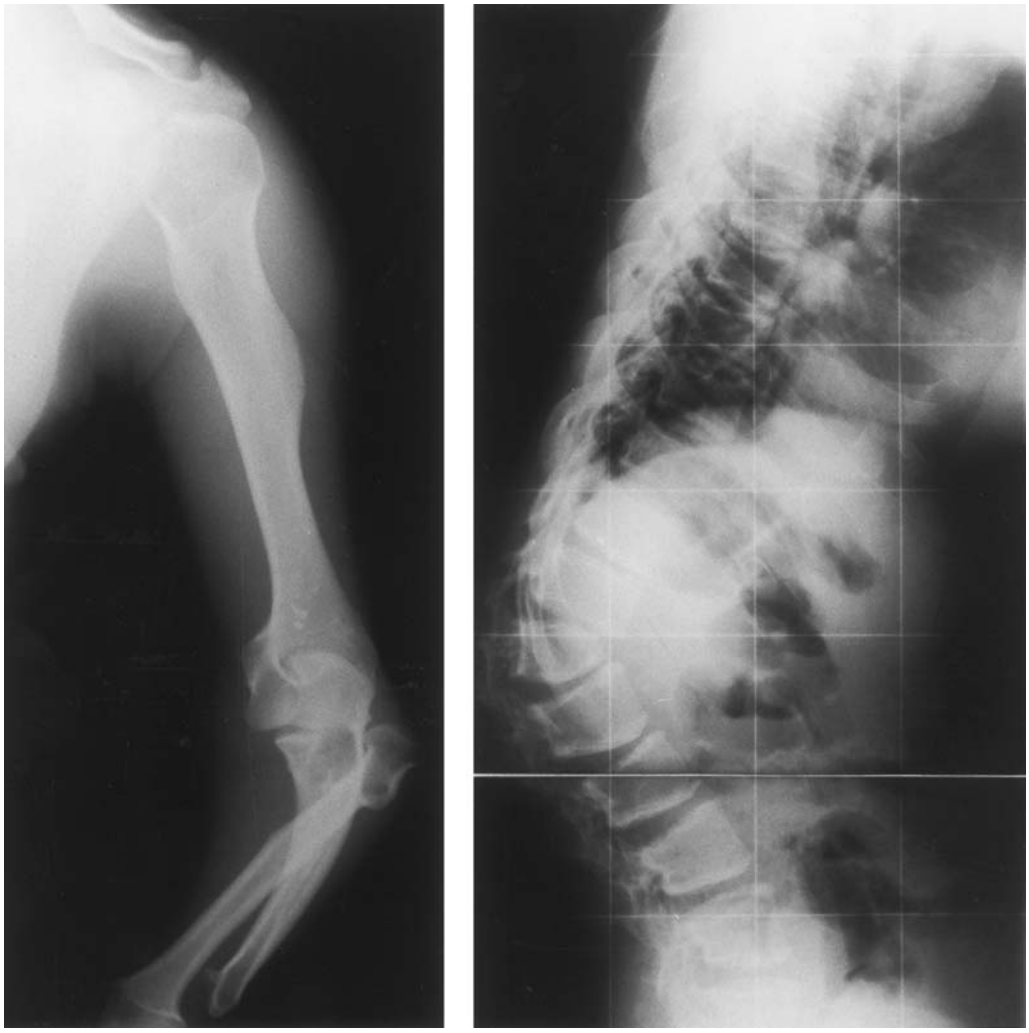

Figure 5 X-rays for individual IV:10. Note shortening of the tubular bones in the upper extremity (*left*), with disproportionate shortening of the radius and ulna in comparison with the humerus; the radial head is also luxated. Lateral X-ray of the spine (*right*) reveals anterior hypoplasia of the first lumbar vertebral body, whereas the second to fifth lumbar vertebral bodies are wedge shaped, with the dorsal margins shorter than the ventral margins.

and those further q telomeric of it, are heterozygous in this affected child, thereby placing them outside the region of presumed homozygosity by descent.

The AMDM candidate interval spans the pericentromeric region of chromosome 9 and is defined by a contiguous series of eight homozygous markers (in the affected children from the consanguineous families) that cover a sex-averaged genetic distance of 6.9 cM, between D9S1853 and D9S1874. Traditional two-point linkage analyses with fully informative markers in this interval yield a LOD score of 5.1 at $\theta = 0$, even without the inclusion of inbreeding loops in the calculations.

Discussion

The specific entity "acromesomelic dwarfism," first reported in three children by Maroteaux et al. (1971), has subsequently been described in >40 individuals (e.g., Langer et al. 1977; Hall et al. 1980; Langer and Garrett 1980; Borrelli et al. 1983; Del Moral et al. 1989). The most comprehensive description of the skeletal features, provided by Langer and Garrett (1980), is based on radiological evaluations of 28 affected individuals. At birth, weight and length may be normal, although limbs may appear short; X-rays reveal short but not malformed appendicular bones. Disproportionate shortening of the limbs becomes more apparent during childhood. In the extreme, radial bowing and posterior dislocation (as in our patient IV:10) may develop. Although all appendicular skeletal elements are short, the distal and middle segments are generally more severely affected than the proximal segment. In the hands, cone-shaped epiphyses are common, and phalanges are short

and broad. Skeletal involvement in AMDM, despite its name, extends beyond the appendicular skeleton in all patients. Oval-shaped vertebral bodies may be radiographically detectable at birth, and other characteristic vertebral changes are clearly recognizable by age 2 years. As with the appendicular features, the vertebral features become more pronounced throughout childhood. The pelvis has a typical configuration, and the cranium may demonstrate frontal bossing, with a normal head circumference. Most affected individuals have no obvious facial dysmorphism; they have normal hair patterns and quantity, normal intelligence, and no evidence for other organ-system involvement.

Although other autosomal recessive skeletal dysplasias having an acromesomelic pattern of involvement have been described (e.g., AMDH and AMDG), most are clinically and radiographically different from AMDM. That AMDH and AMDG are clinically, radiologically, and etiologically different from AMDM is now confirmed by their genetic mapping to different loci. Mutations in the transforming growth factor–β family member CDMP1, on chromosome 20, have been reported in both AMDH and AMDG (Thomas et al. 1996, 1997). We excluded linkage to CDMP1 in the Maroteaux type and then mapped the actual AMDM candidate interval to chromosome 9p13-q12. It will be interesting to determine whether other as yet unmapped acromesomelic dysplasias, or other heritable disorders having predominantly mesomelic or acromelic involvement, will be linked to the AMDM locus.

The AMDM candidate interval overlaps with the candidate interval for another autosomal recessive osteochondrodysplasia, cartilage-hair hypoplasia (Sulisalo et al. 1994). Although both disorders are associated with disproportionate limb involvement, their clinical and radiographic differences suggest they are likely to be etiologically different rather than allelic variants. With >150 distinct skeletal dysplasias having been described, it is not surprising that genes responsible for causing two different skeletal dysplasias will map near each other. Minty and Hall (1993) reported two siblings of consanguineous parents who had both AMDM and familial hypomagnesemia (HRH). The authors noted that the siblings' concordance for these two autosomal recessive conditions suggests that the disorders could be linked. Our mapping of AMDM to chromosome 9p13-q12, coupled with the mapping of HRH to 9q12-21 (Walder et al. 1997), confirms this linkage. At present, only one candidate gene for AMDM, the interleukin 11 receptor (IL11RA), which is expressed in skeletal progenitor cells during murine development, has been placed within the 6.9-cM candidate interval (Neuhaus et al. 1994; Povey et al. 1997); no other likely candidate genes or homologous murine phenotypes have been mapped to the orthologous chromosomal regions on mouse chromosomes 4 and 19 (Pilz et al. 1995).

A single case report detailing light-microscopic findings in an iliac crest biopsy from a 51-year-old female patient with AMDM has been published (Del Moral et al. 1989); the occurrence of fibrous cartilage within bony trabeculae suggested an abnormality in membranous ossification. However, because of the patient's age, the process of endochondral ossification (the more likely site of abnormality in our opinion) could not be evaluated. At present, insufficient information is available to speculate on the type of gene product likely to be abnormal in AMDM. Linkage data clearly suggest that a locus responsible for causing AMDM is on human chromosome 9. Both refinement of the candidate interval and testing for locus homogeneity will require the study of additional families.

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Electronic-Database Information

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