*Am. J. Hum. Genet. 65:175–182, 1999*

# A Gene for Autosomal Recessive Spondylocostal Dysostosis Maps to 19q13.1-q13.3

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## Summary

**In spondylocostal dysostosis (SD), vertebral-segmentation defects are associated with rib anomalies. This results in short-trunk short stature, nonprogressive kyphoscoliosis, and radiological features of multiple hemivertebrae and rib fusions. SD can be familial, and both autosomal dominant and autosomal recessive (AR) inheritance have been reported, but no genes have been identified or localized for nonsyndromic SD in humans. We performed genomewide scanning by homozygosity mapping in a large consanguineous ARSD Arab Israeli family with six definitely affected members. Significant linkage was found to chromosome 19q13, with a LOD score of 6.9. This was confirmed in a second Pakistani family with three affected members, with a LOD score of 2.4. The combined-haplotype data identify a critical region between D19S570 and D19S908, an interval of 8.5 cM on 19q13.1-19q13.3. This is the first study to localize a gene for nonsyndromic SD. ARSD is clinically heterogeneous and is likely to result from mutations in developmental genes or from regulating transcription factors. Identification of these genes will improve the understanding of the molecular processes contributing to both normal and abnormal human vertebral development.**

## Introduction

Spondylocostal dysostosis (SD; MIM 277300) is the association of multiple vertebral-segmentation defects with rib anomalies. This results in a short-trunk short-stature syndrome, with nonprogressive kyphoscoliosis and radiological appearances of a variable degree of multiple hemivertebrae. The involvement of the thoracic spine leads to irregular rib alignment and points of fusion, and occasionally ribs are absent. SD can be either sporadic (Mortier et al. 1996) or familial. Monogenic SD families have been reported, with both autosomal dominant (AD) (Rimoin et al. 1968; Temple et al. 1988; Floor et al. 1989; Lorenz and Rupprecht 1990) and autosomal recessive (AR) inheritance (Norum and McKusick 1969; Cantú et al. 1971; Castroviejo et al. 1973; Franceschini et al. 1974; Silengo et al. 1978; Beighton and Horan 1981; Turnpenny et al. 1991; Satar et al. 1992). Associated features in familial SD include anal and urogenital anomalies (Casamassima et al. 1981), congenital heart disease (Delgoffe et al. 1982; Aurora et al. 1996), limb abnormalities (Wadia et al. 1978), plagiocephaly-torticollis sequence (Turnpenny et al. 1991), and inguinal hernias in male patients (Bonaime et al. 1978; Turnpenny et al. 1991), but with no neurological signs or mental retardation. In sporadic cases, clinical features are very heterogeneous, and malformations beyond the vertebrae and ribs are more common (Kozlowski 1984; Martínez-Frías et al. 1994; Mortier et al. 1996).

Terminology in the literature for anomalies of spinal development is potentially confusing because multiple names have been used for similar clinical syndromes. "Spondylothoracic dysostosis" has been used for the severe and often lethal AR form, which presents a crablike chest on radiograph—the Jarcho-Levin syndrome (Jarcho and Levin 1938; Aymé and Preus 1986)—but also sometimes has been used for the mild AR form (similar to the phenotypes seen in families of this study). "Spondylocostal dysostosis" has been reserved by some (Mortier et al. 1996) for AD families, in which the phenotype is generally mild, but has also been used for the mild AR form (Roberts et al. 1988). The term "costovertebral dysplasia" features in earlier reports (Noram and McKusick 1969; Cantú et al. 1971; Bartsocas et al. 1974; David and Glass 1983).

Vertebral-segmentation defects are a feature of numerous syndromes and genetic conditions, including

Received February 12, 1999; accepted for publication April 29, 1999; electronically published June 7, 1999.

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0002-9297/99/6501-0023$02.00

Klippel-Feil syndrome, Goldenhar syndrome, X-linked dominant incontinentia pigmenti, and the VATER (vertebral defects, anal atresia, tracheo-esophageal fistula, radial defects and renal anomalies) and VACTERL (vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, radial defects and renal anomalies, nonradial limb defects) associations. Vertebral anomalies have also been associated with chromosome abnormalities (Crow et al. 1997; Dowton et al. 1997), including trisomy 8 mosaicism (Riccardi 1977) and an apparently balanced 14;15 chromosome translocation (De Grouchy et al. 1963).

The genes and genetic loci for nonsyndromal SD are unknown. Genes have been cloned for two monogenic conditions that include vertebral-segmentation defects as a feature: Alagille syndrome (*jagged-1,* chromosome 20p12) and Simpson-Golabi-Behmel syndrome (*glypican 3,* chromosome Xq26). In both cases the vertebral abnormalities are minor compared with those associated with SD, which is therefore phenotypically very different. The closest animal models to SD are the *undulated* mouse, in which vertebral abnormalities occur because of mutations in *Pax-1* (Balling 1994), and the pudgy (*pu*) homozygous mouse, in which vertebral abnormalities occur because of mutations in *Dll3* (Kusumi et al. 1998). The human homologues for *Pax-1* and *Dll3* are located on chromosomes 20p11.2 and 19q13.2-q13.3, respectively (Giampietro et al. 1999). To date, no human disease associated with mutations in *Pax-1* is known, and mutations have not been found in cases of Klippel-Feil syndrome (Smith and Tuan 1994).

The availability of a large inbred family with ARSD, first reported by Turnpenny et al. (1991), has made it possible to begin to elucidate the molecular genetics of SD in humans by homozygosity mapping using a DNA-pooling strategy and genomewide screening. An additional consanguineous SD family has also been recruited. Their phenotypes are described and illustrated, and the results of linkage analysis are presented.

## Families and Methods

### *Families*

We performed genomewide scanning by homozygosity mapping in a large consanguineous ARSD Arab-Israeli family with six definitely affected members. An additional consanguineous SD family was also recruited. The pedigrees of family 1 and family 2 are shown in figures 1 and 2, respectively.

*Family 1.*—Family 1 is a large inbred Arab-Israeli kindred with six living affected individuals and one deceased individual in three disparate branches, first reported by Turnpenny et al. (1991) (fig. 1). The family is highly consanguineous, but the exact relationships to a single common ancestor are no longer known. The chest radiographs of the index case (individual VI-1) at age 2.5 years and of an affected adult at age 31 years (individual V-18) are shown in figure 3*A* and *B,* respectively. Involvement of the spine in all the affected cases is extensive, and there is consistency in the malsegmentation pattern, throughout the length of the vertebral column. Most of the affected male subjects have unilateral or bilateral inguinal hernias, which have required surgical repair. They had no neurological signs, and renal ultrasound in all cases studied was normal. The family was revisited and blood samples, for DNA extraction, were taken from the 6 living affected cases, 9 obligate carriers, and 13 individuals at high-carrier risk. The affection status of a seventh individual, V-8, was uncertain because of very mild vertebral abnormalities, consisting of mid-thoracic scoliosis (fig. 3*C*) without segmentation defects and lower-lumbar spinal abnormality (fig. 3*D*), apparently with a segmentation defect. She showed a plagiocephaly-torticollis sequence, and the clinical impression was of an individual likely to be homozygous for the putative gene mutation but demonstrating a very mild phenotype.

*Family 2.*—Family 2 (fig. 2) is a nuclear consanguineous family originating from the Rawalpindi area of Pakistan. They are believed to be descended from Moghuls 600 years ago and have no known connections with migrations of Arabs from the Middle East. They consist of second-cousin parents with three affected and two unaffected children. Their phenotype is very similar to that of family 1 (fig. 3*E*), with the most severe segmentation defects, in individual V-5, seen in the thoracic region. Clinically, the affected individuals manifest a short-trunk form of dwarfism. The affected male subject did not have hernias, and renal ultrasonography has not been performed.

### *Initial Genomewide Screen*

To rapidly screen for markers linked to the disease, we adopted an initial DNA-pooling strategy. This strategy takes advantage of the likelihood that affected individuals in inbred families share a common chromosomal region inherited from a common ancestor (Lander and Botstein 1987). A pool of DNA samples from the seven affected members of family 1 were used as templates for the amplification of genomewide markers. With use of this approach, markers linked with the disease phenotype show a shift in allele frequencies, toward a single homozygous allele in the affected DNA pool.

### *Pooling of DNA*

DNA was extracted from samples of peripheral blood, by standard methods. The DNA concentrations were determined by spectrophotometric measurements at OD260. DNA samples were diluted to 50 ng/$\mu$l, and individual DNA samples from the affected members of

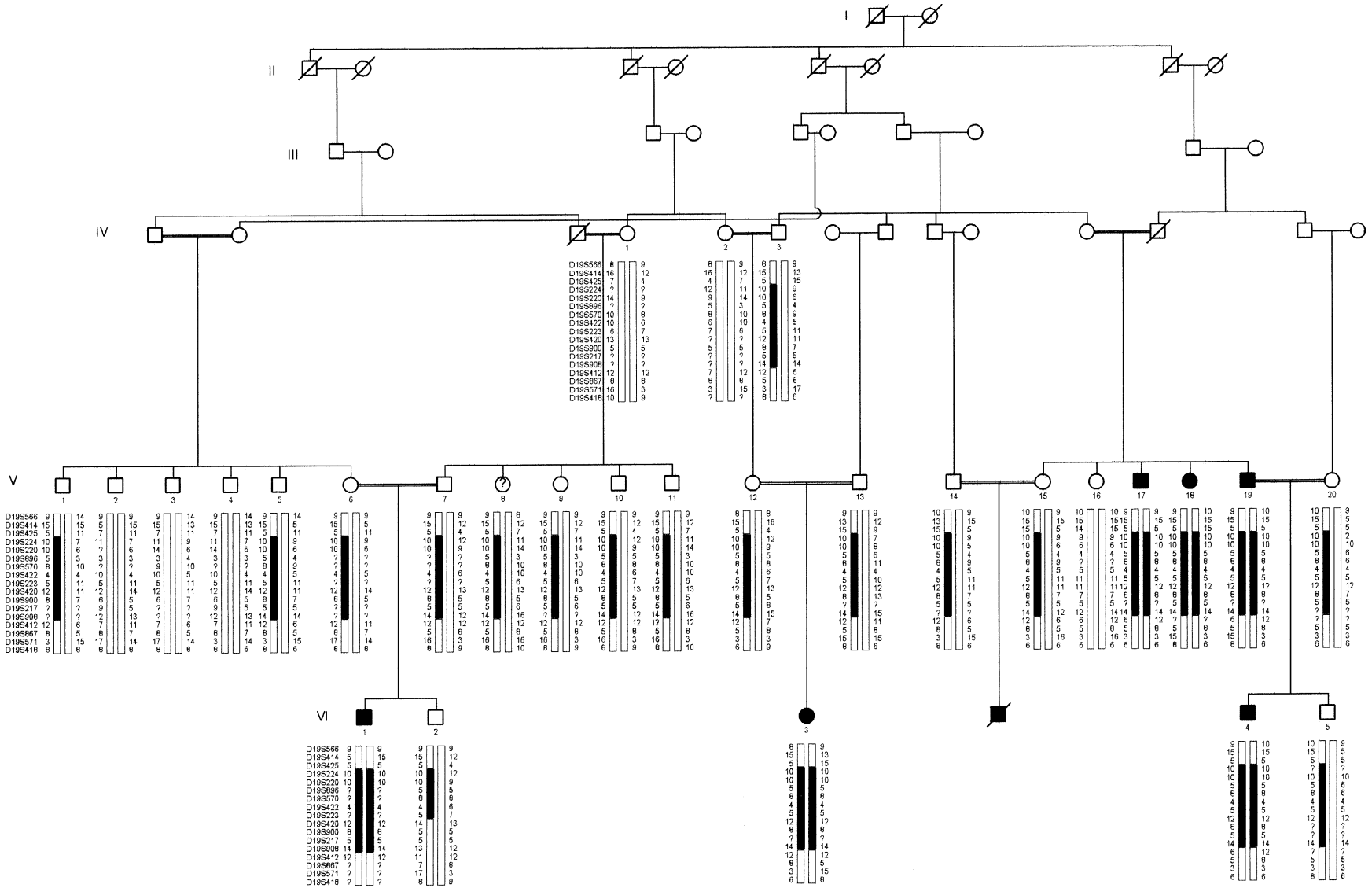


**Figure 1** Pedigree of family 1, with genotypes for 17 DNA markers from chromosome 19q13. A question mark (?) within a haplotype indicates genotypes that were not determined. Regions of homozygosity are indicated by blackened bars. Affected and unaffected individuals are represented by blackened and unblackened symbols, respectively. The uncertainty about the affected status of individual V-8 is indicated by a question mark (?). Double lines indicate consanguineous marriages. The precise relationships of generations I–III are no longer known; the simplest ancestry is therefore shown.

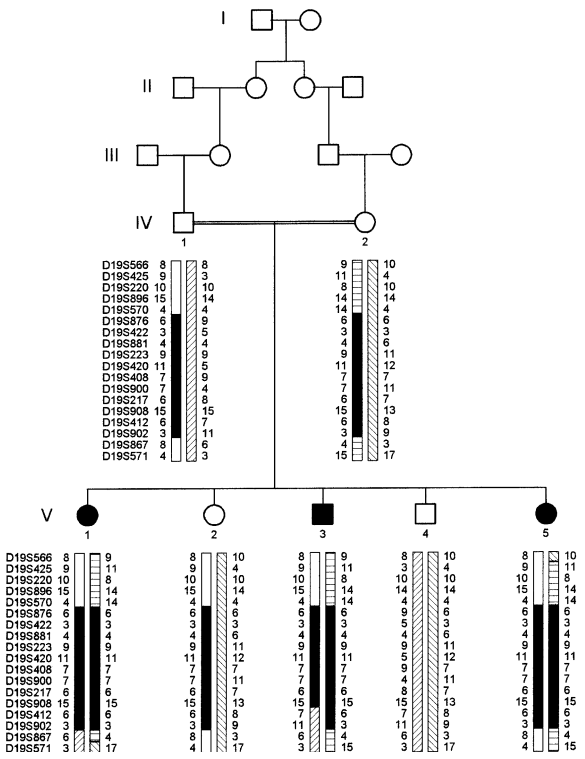


**Figure 2** Pedigree of family 2, with genotypes for 18 DNA markers from chromosome 19q13. Regions of homozygosity are indicated by blackened bars. Affected and unaffected individuals are represented by blackened and unblackened symbols, respectively. Double lines indicate consanguineous marriages.

family 1 were tested in PCR assays to demonstrate equal amplification between samples prepared from different individuals. The affected pool from family 1 consisted of seven DNA samples (from individuals V-8, V-17, V-18, V-19, VI-1, VI-3, and VI-4) at a final concentration of 50 ng/$\mu$l.

### *Genotyping*

Pooled DNA from the seven affected individuals from family 1 was used for the initial genome screen. This was performed with 343 autosomal markers, from the Perkin-Elmer Biosystems Linkage Mapping Set (version 1). These markers are spaced at ~10-cM intervals and have an average heterozygosity of .8. Forward primers are labeled with either 6-FAM, HEX, or TET fluorescent dyes, which allows multiplex analysis to be done on an ABI 377 DNA sequencer. The reverse primers have a 5′GTTT "PIGtail," which has been shown to facilitate >85% addition of the nucleotide adenosine to the 3′ end of the forward product (Brownstein et al. 1996). PCR reactions were performed with 75 ng pooled DNA in a 7.5-$\mu$l PCR reaction mixture containing 2.5 pmol each primer, 200 $\mu$M each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, and 0.3 U Ampli*Taq* Gold (Perkin-Elmer Applied Biosystems). Reactions were performed in 96-well microtiter plates, and amplification was performed on a Perkin-Elmer model 9600, with an initial denaturation step of 12 min at 95°C; this was followed by 10 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 30 s; then by 20 cycles of 89°C for 15 s, 55°C for 15 s, and 72°C for 30 s; and then by 72°C for 10 min. PCR products were pooled in a 1:2:1 ratio of 6-FAM:HEX:TET–labeled products and then were mixed with formamide, GS-500 size standard (Perkin-Elmer Applied Biosystems), and Blue dextran (Perkin-Elmer Applied Biosystems). The products were separated, by electrophoresis, onto 4% (w/v) denaturing polyacrylamide gels, on an ABI 377 DNA sequencer. GENESCAN ANALYSIS 2.1 and GENOTYPER 2.0 software were used to size PCR products and analyze data.

The affected subjects were analyzed separately for all markers at which two or more alleles in the pooled sample were not clearly identified. When individual analysis of affected individuals showed widespread homozygosity, the whole family was analyzed, to establish whether there was homozygosity by descent with carriers being heterozygous.

### *Confirmation of Region of Homozygosity*

After a potential region of homozygosity was identified, additional markers were selected from the Généthon. This region was then tested in both families. Haplotype analysis in these linked families was done to define the localization of the gene.

### *Linkage Analysis*

Two-point linkage analysis, between affected status and the haplotype apparently segregating with the disease, was done with the MLINK program (version 5.2) of the LINKAGE package. For these analyses, an AR mode of inheritance and a disease-allele frequency of .0001 were used. The frequency of the haplotype apparently segregating with the disease was assumed to be .1; this represents a highly conservative estimate, given the number (10) of markers homozygous in this region and their informativity (~0.8% heterozygosity).

## Results

### *Initial Screen*

In the initial screen of pooled DNA from family 1, two or more alleles were not clearly identified for 44 of the 343 markers. These 44 markers were investigated further by genotyping the individual DNA from the seven affected members of family 1. The seven affected subjects were not homozgous for a single allele, with any marker. However, six of the seven affected individ-

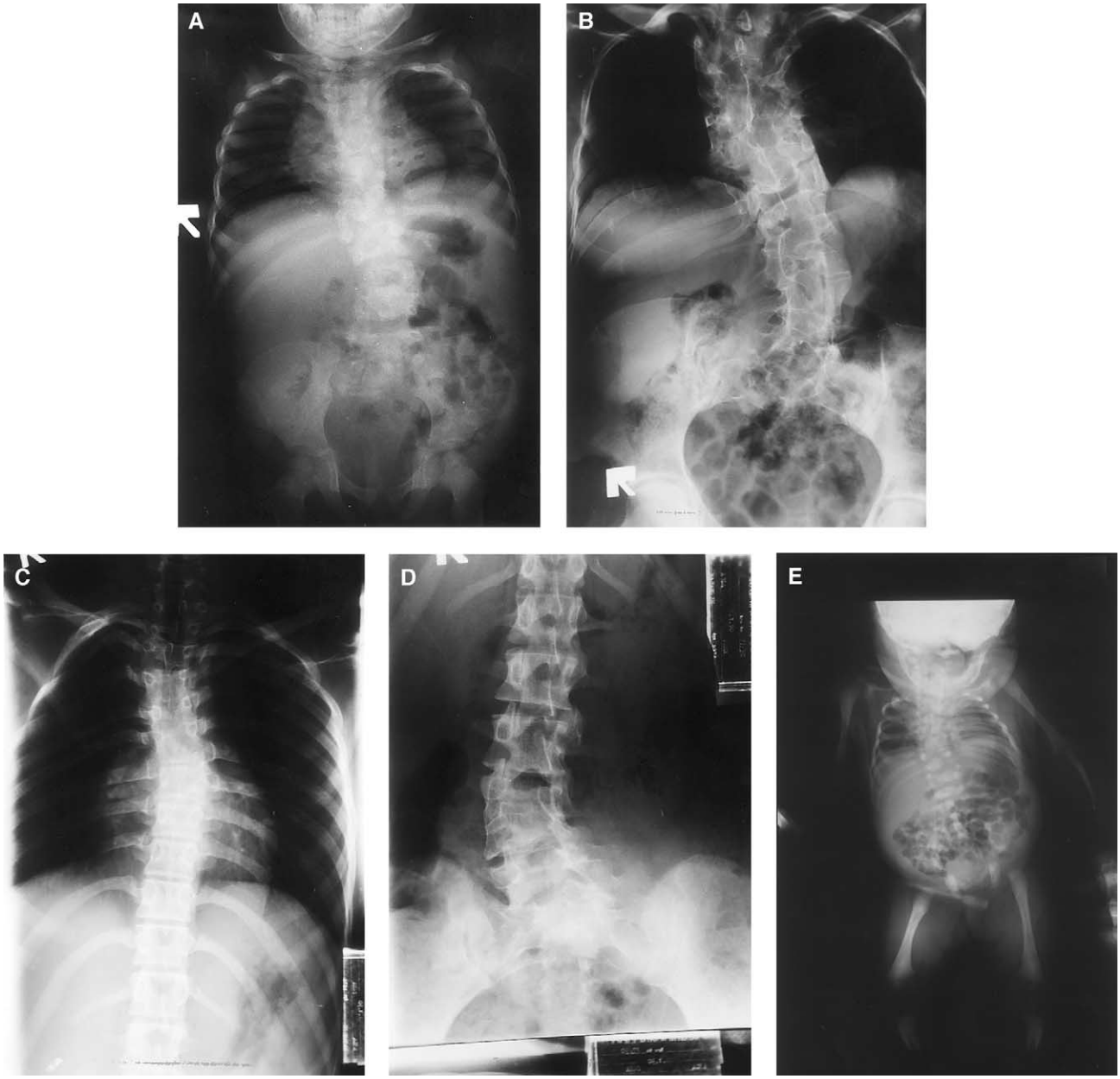


**Figure 3** *A,* Radiograph of the spine of individual VI-1 (family 1), at age 2.5 years. Extensive malsegmentation of the vertebrae throughout the spinal column is present, with scoliotic curves. There are 11 pairs of ribs and multiple points of fusion. *B,* Radiograph of the spine of individual V-18 (family 1), at age 31 years. Extensive malsegmentation is present, giving rise to a nonprogressive scoliotic deformity. *C,* Radiograph of the thoracic spine of individual V-8 (family 1), at age 15 years. Two mild scoliotic curves are present, apparently without a segmentation defect. There are no points of rib fusion. *D,* Radiograph of the lumbar spine of individual V-8 (family 1), at age 15 years. Segmentation defects appear to affect L4–S1. *E,* Radiograph of individual V-5 (family 2), in the neonatal period. Vertebral-segmentation defects are most severe in the cervical and thoracic regions.

uals were homozygous for five markers (D6S462, D10S548, D14S276, D14S78, and D19S220). The unaffected family members were then genotyped for these five markers, to identify linkage to the disease phenotype rather than homozygosity due to the presence of common alleles. D19S220 was the only marker that showed homozygosity by descent in all affected individuals—except for V-8, who shows a markedly milder phenotype compared with those in her affected relatives. Individual V-8 and all obligate carriers were heterozygous for D19S220. Additional markers close to D19S220 were typed, and a homozygous region was identified between D19S425 and D19S412 (fig. 1). The distance between these markers is 11.2 cM. A maximum LOD score of

8.9 at a recombination fraction of 0 was calculated, with the assumption that individual V-8 is of "unknown" affected status. We obtained this value by dividing the family into two separate parts and summing the two resulting LOD scores, since the presence of multiple consanguineous loops imposes computational limits. This value therefore represents a conservative estimate. As expected, the LOD score was sensitive to the given haplotype frequency, although the evidence for linkage in this family remained strong for a wide range of frequencies—LOD scores of 5.9, 6.9, and 8.9 were obtained for linked-allele frequencies of .2, .1 and .01, respectively.

### *Confirmation of Linkage to 19q*

Linkage of SD to the candidate region on chromosome 19q was confirmed in family 2. The three affected subjects (V-1, V-3, and V-5) of family 2 were homozygous for markers D19S876–D19S902 (fig. 2). The LOD score in this family was 2.4 with a haplotype frequency of .1 and was 3.0 with a frequency of .01. The linked haplotype was different from that of family 1. Haplotype analysis of the two families allowed the genetic interval to be narrowed to an 8.5-cM region, between markers D19S570 and D19S412 (fig. 4).

## Discussion

We have localized the first gene for nonsyndromic SD, using homozygosity mapping. We have shown significant linkage, in two unrelated pedigrees with ARSD, to an 8.5-cM region on chromosome 19q13.1-q13.3.

DNA linkage mapping by homozygosity of descent in inbred kindreds with AR conditions is now a well-established strategy for the localization of genes causing distinctive phenotypes or disease (Lander and Botstein 1987; Gelb et al. 1995). It is especially powerful when multiple affected individuals are present in disparate branches of a large kindred, such as in family 1 in this study. In genomewide screening, only one region of homozygosity was identified, in all six clearly affected individuals, with obligate carriers demonstrating heterozygosity. Individual V-8, with mild vertebral changes (fig. 3*B* and *C*), appears to be a heterozygous carrier. Since all obligate carriers, when studied, had radiologically normal spines, her scoliosis may have a different etiology, or it may be that she is a manifesting carrier for ARSD.

In family 2 there was also linkage to the 19q13 locus, with a significant LOD score independent of that in family 1. Haplotype analyses for these two families confirm that a gene for ARSD lies between markers D19S570 and D19S412, a critical region of 8.5 cM. Distinct haplotypes in the region of interest in these two families refutes the possibility of a founder effect—which is not surprising given their ethnic diversity.

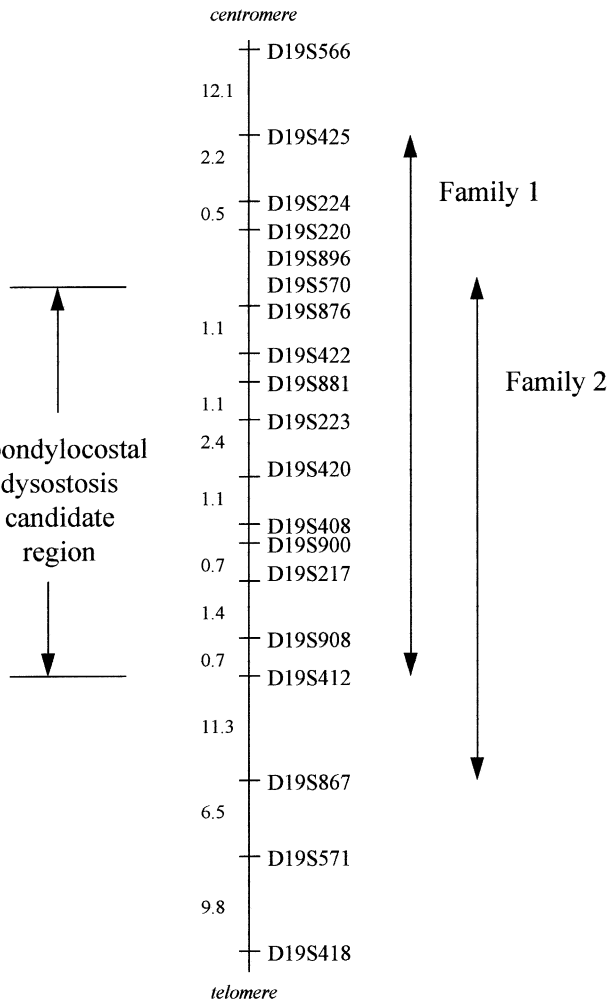


**Figure 4** Genetic map and distances of chromosome 19 markers. The location of the SD gene is determined on the basis of the region of homozygosity in families 1 and 2.

The nature of the phenotype in SD suggests an alteration in either a developmental gene or a regulating transcription factor. In humans, vertebrae develop, at 3.5–6 wk of embryonic life, from the somites, after differentiation into dermatome, myotome, and sclerotome. Sclerotomic cells spread out to form a perichordal sheath. Dense and loose areas develop in both the sclerotome and perichordal sheath, from which the central dense area gives rise to the intervertebral disks, the peripheral dense area gives rise to the neural process of the vertebra and rib, and the loose central zone forms the vertebral column. Altered regulation of cell signaling, during what is normally an ordered and precise sequential process of cellular differentiation, presumably gives rise to irregularly shaped vertebrae, or hemivertebrae,

and rib anomalies. Although these processes must be subject to a variety of developmental insults, as evidenced by the range of distinct syndromes causing segmentation defects, the relative paucity of associated malformations in the monogenic forms of SD suggests an alteration, in gene expression, that is very localized and time limited. It is logical to implicate elements within the equivalent of the "vertebral column *hox* code," which has been elucidated in the mouse (Kessel 1991; Horan et al. 1995), or, possibly, within other developmental genes, such as *pax* or *pou*. Chromosome 19q13 is a gene-rich region of the genome, which contains a large number of expressed sequence tags (ESTs): 273 ESTs between markers D19S228 and D19S219, according to the GB4 map (GeneMap). However, the identification of *Dll3* in the pudgy homozygous mouse (Kusumi et al. 1998), whose human homologue is located at 19q13.2-q13.3 (Giampietro et al. 1999), makes this a strong candidate gene. Pudgy mice have severe vertebral and rib deformities, thus demonstrating a phenotype that is close to ARSD in humans. *Dll3* is homologous to the *Notch*-ligand *Delta* in *Drosophila melanogaster* and is involved both in the developmental integrity of early somite formation and the patterning of vertebrate paraxial mesoderm. Identification of the gene(s) and disease-causing mutations in families with Mendelian forms of SD will ultimately lead to an improved understanding of the molecular processes that give rise to normal and abnormal development of the spine.

## Acknowledgments

We are grateful for the cooperation of the families studied and for the support of the Skeletal Dysplasia Group (United Kingdom), the Children's Research Fund, the Darlington Charitable Trust, the Royal Devon and Exeter National Health Service Healthcare Trust, and the University of Exeter. We acknowledge the excellent technical assistance of Martin Goddard. The continued support from Mark Dronsfield and Perkin-Elmer Biosystems is appreciated.

## Electronic-Database Information

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

GeneMap, http://www.ncbi.nlm.nih.gov/genemap/map.cgi?chr=19 (for ESTs within homozygous region on chromosome 19)

Généthon, http://www.genethon.fr/genethon_en.html (for additional markers for chromosome 19 region)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for SD [MIM 277300])

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