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Localization of a Multiple Synostoses–Syndrome Disease Gene to Chromosome 17q21-22

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Summary

Multiple synostoses syndrome is an autosomal dominant disorder characterized by premature onset of joint fusions, which initially affect the interphalangeal joints, by characteristic facies, and by deafness. We performed linkage analysis on a large Hawaiian family with multiple synostoses syndrome. Because another autosomal dominant disorder, proximal symphalangism, shares some clinical symptoms with multiple synostoses syndrome and has been linked to markers at loci at chromosome 17q21-22, we tested the hypothesis that multiple synostoses syndrome is linked to the same chromosomal region. Using polymorphic markers from the proximal symphalangism interval, we conducted linkage analysis and showed that the multiple synostoses–syndrome phenotype is linked to the same chromosomal region. A maximum LOD score of 3.98 at recombination fraction of .00 was achieved for the marker at locus D17S787. Further genetic analysis identified individuals with recombinant genotypes, allowing localization of the disease gene within the interval D17S931–D17S792, a 16-cM region. These data provide evidence that multiple synostoses syndrome and proximal symphalangism may be allelic disorders.

Introduction

Multiple synostoses syndrome (OMIM 186400 and 186500) is a rare autosomal dominant dysostosis primarily characterized by premature joint ankylosis, or fusion. The term "multiple synostoses syndrome" was first coined by Maroteaux et al. (1972). Numerous other synonyms and eponyms have been assigned to this disorder, including WL syndrome, deafness of Herrmann, and facio-audio-symphalangism (Furhmann et al. 1966; Herrmann 1974; Hurvitz et al. 1985). In addition to the ankylosis, affected individuals have characteristic facial manifestations that include a broad hemicylindrical nose, with lack of alar flare, and a thin upper vermilion. Most affected individuals also develop early-onset otosclerotic deafness that typically responds to stapedectomy (Gaal et al. 1987).

In multiple synostoses syndrome, joint ankylosis begins in early childhood and is progressive. The fifth proximal interphalangeal joint is usually the first to be affected, and the ankylosis proceeds in an ulnar-to-radial and proximal-to-distal direction, typically involving digits 3, 4, and 5. The carpal and tarsal bones, the radius, and the humerus are also involved. Other findings include clinodactyly, brachydactyly, a shortened hallux, syndactyly of toes 2 and 3, and mild short stature. Radiographs document the natural history of the disease, by demonstration of multiple areas of progressive joint-space narrowing and resultant joint fusions.The interphalangeal joint spaces begin to narrow at an early age, and phalangeal bone fusions develop subsequently. Frequently, the vertebral, humeroradial, and hip joints are simultaneously involved.

Multiple synostoses syndrome shares clinical similarities with proximal symphalangism (SYM1 [OMIM 185800]). Both disorders present with early onset and progressive symphalangism, and some patients with SYM1 manifest conductive hearing loss (Strasburger 1965). Polymeropoulos et al. (1995) localized the disease gene for SYM1 to an ~30-cM interval, flanked by the loci D17S579 and D17S795, on chromosome 17q21-22. Using a single large Hawaiian family with multiple synostoses syndrome, we tested the hypothesis that the multiple synostoses–syndrome disease gene is located in the SYM1 gene region. Linkage analyses of these data provided strong evidence that the disease gene for mul-

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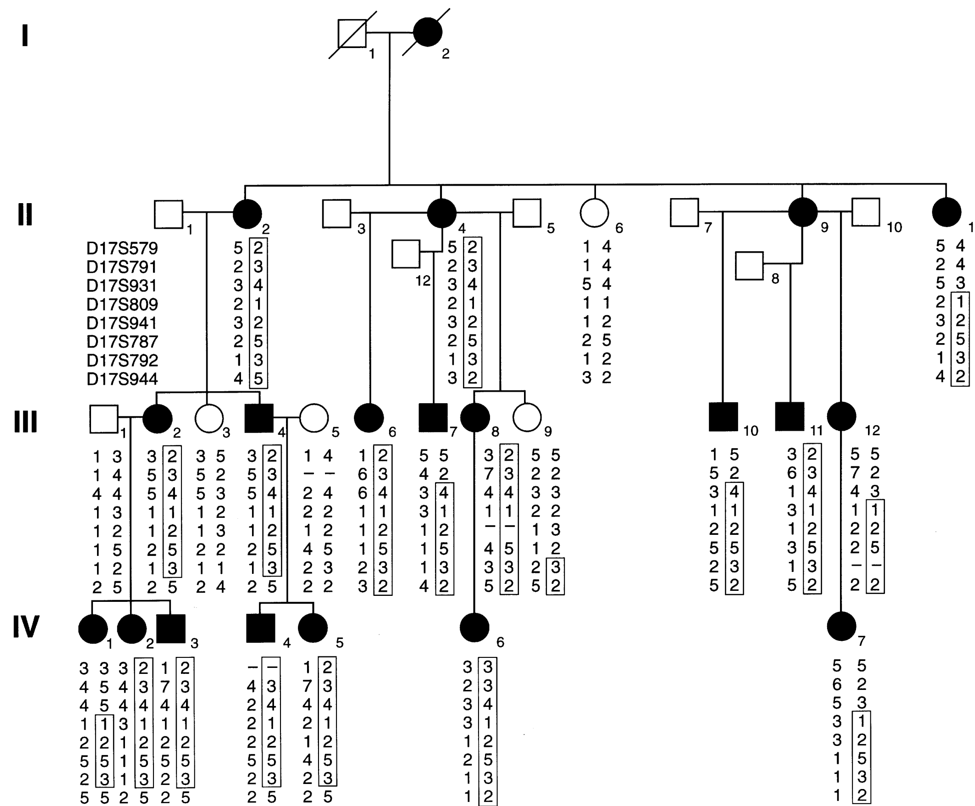

Figure 1 Pedigree of the Hawaiian family with multiple synostoses syndrome, showing the most likely haplotypes for the chromosome 17 markers. The haplotype linked to multiple synostoses syndrome is boxed.

tiple synostoses syndrome is within a 16-cM interval at 17q21-22 that is completely contained within the SYM1 interval, suggesting that the two disorders are allelic.

Material and Methods

Ascertainment

Each of 25 family members who agreed to participate was examined by one of us (D.K., D.L.R., B.P., or K.R.). Individuals were scored as affected if they had evidence of limited flexion of the fifth proximal interphalangeal joint and/or fusions of multiple other joints. When appropriate, we obtained radiographic evidence of symphalangism or other joint fusions, to confirm the diagnosis. Clinical status could be determined unequivocally for all participating family members. The study was approved by the hospital's institutional review board.

DNA Typing

DNA was extracted from blood by use of the Puregene kit (Gentra). Polymorphic short tandem-repeat markers from the SYM1 region on 17q21-22 were applied to this family. Linkage refinement was conducted with markers from the Whitehead Institute for Biomedical Research (Hudson et al. 1995), the Cooperative Human Linkage Center (Murray et al. 1994), and Généthon (Dib et al. 1996). All primer sequences are available through either the Genome Database or the Whitehead Institute. Primers were obtained from Research Genetics. Fragments containing the microsatellites were amplified by PCR, the products were separated on 6% denaturing polyacrylamide gels, and the alleles were scored by size.

Linkage Analysis

Linkage analysis was performed by 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. The phenotype was coded as a fully penetrant autosomal dominant trait with a disease-allele frequency of .0001. Equal recombination frequencies for males and females were assumed. Because allele frequencies in this Hawaiian population are unknown, for each marker, the allele frequencies were set at $1/N$, where N is the number of observed alleles in the pedigree.

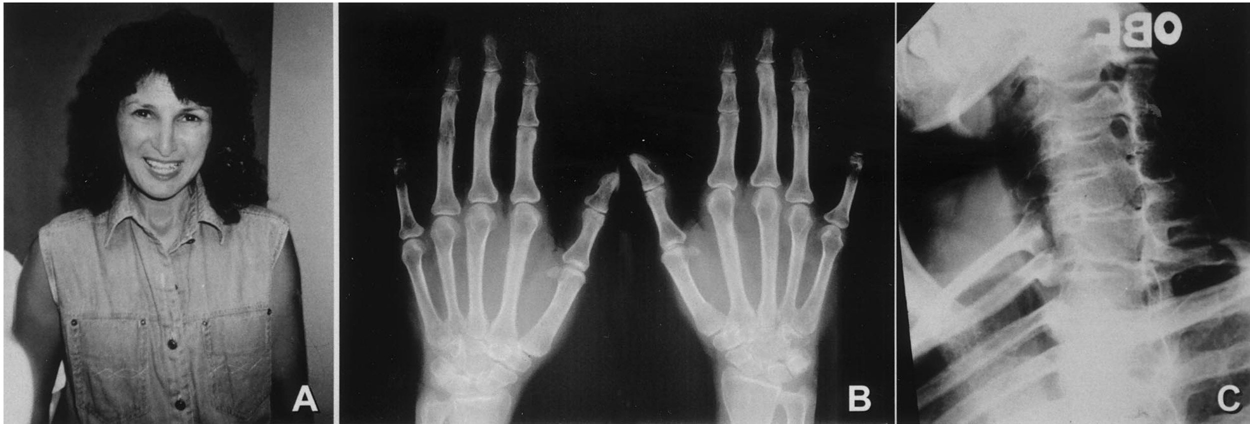

Figure 2 Composite of the proband. *A,* Characteristic facial findings in multiple synostoses syndrome, including long, narrow facies; broad tubular nose, with lack of alar flare; and thin upper vermilion. *B,* Adult hand radiograph demonstrating symphalangism with significant narrowing of the proximal interphalangeal joint of the fifth digit and progressive symphalangism of the middle interphalangeal and distal interphalangeal joints of digits 3, 4, and 5. *C,* Recent lateral radiograph of the proband, showing cervical vertebral fusions of vertebrae 3, 4, 5, and 6.

Results

Clinical Presentation

A large Hawaiian family (International Skeletal Dysplasia Registry, reference R95-127) with multiple synostoses syndrome was initially identified by Gaal et al. (1987). The family can trace the disorder back to the first known affected individual, a Cherokee Indian who arrived in the Hawaiian Islands in the 1870s. A partial pedigree, showing the individuals studied in this investigation, is shown in figure 1. Affected individuals demonstrated the cardinal features of the syndrome, including a broad, tubular-shaped nose, otosclerotic deafness, and multiple progressive joint fusions commencing in the hand (fig. 2). All affected individuals examined have some degree of hearing impairment, ranging from mild to severe. Symphalangism was the most consistent finding. The joint fusions were progressive, commencing in the fifth proximal interphalangeal joint in early childhood (or at birth, in some individuals) and progressing in an ulnar-to-radial and proximal-to-distal direction. Most of the affected adults were unable to close their hands into a fist. With increasing age, ankylosis of other joints—including the cervical vertebrae, hips, and humeroradial joints—develops. Many reports in the literature (Furhmann et al. 1966; Herrmann 1974; Hurvitz et al. 1985) note the humeroradial and tarsal involvement; however, little has been written regarding the vertebral bodies. In the family reported here, the cervical vertebral fusions commence in early childhood and ultimately produce significant limitation of neck flexion and extension.

Linkage Analysis

Since multiple synostoses syndrome shares clinical findings with SYM1, a disorder localized to chromosome 17q21-22 (Polymeropoulos et al. 1995), polymorphic markers from the SYM1 region were applied in this family. The SYM1 region on chromosome 17 was defined by D17S579 on the centromeric limit and by D17S795 on the telomeric boundary (see fig. 3), an interval of ~30 cM (Polymeropoulos et al. 1995). Initially, a suggestive LOD score of 2.3 at recombination fraction (θ) of .00 was obtained for the marker at locus D17S797, and, subsequently, a LOD score of 3.98 (θ = .00) was calculated for the marker at locus D17S787. Two-point LOD scores for additional markers from the region are given in table 1.

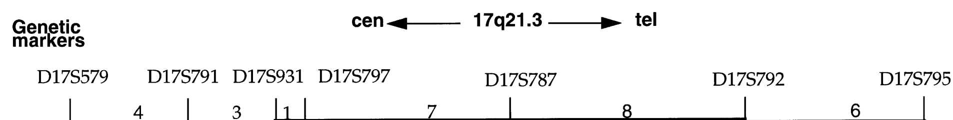

Figure 3 Map of the multiple synostoses–syndrome gene region on chromosome 17q21-22. Genetic distances between the markers are given in centimorgans. The bold line represents the multiple synostoses–syndrome interval based on recombination mapping.

Table 1

Two-Point LOD Scores between Multiple Synostoses Syndrome and Markers at Loci on Chromosome 17

	LOD Score at θ =						
Locus	.00	.01	.05	.10	.20	.30	.40
D17S579	$-\infty$	−12.3	−2.42	−.96	.08	.31	.19
D17S791	$-\infty$	−5.75	−1.86	−.45	.51	.65	.42
D17S797	2.31	2.27	2.10	1.88	1.41	.93	.43
D17S787	3.98	3.92	3.68	3.34	2.57	1.68	.73
D17S792	$-\infty$	2.27	2.68	2.61	2.10	1.36	.53
D17S944	$-\infty$	−1.17	.72	1.31	1.52	1.22	.63

Haplotypes were constructed by parsimony and are shown with the pedigree in figure 1. Four key recombinant events were identified, which allowed the interval containing the disease gene to be narrowed. In individuals II-11, III-12, and IV-l, a recombination between the markers at loci D17S931 and D17S809 established the centromeric limit of the disease-gene region at D17S931. This recombination was confirmed by the haplotype of IV-7, the daughter of III-12, who inherited the recombinant chromosome. The genetic locations for the markers at loci D17S931 and D17S797 are estimated to be similar. For the haplotype analysis, D17S931 was used because of the informativeness of the marker, compared with D17S797, at that locus. A recombination event in individual III-9, between the markers at loci D17S792 and D17S944, defined the telomeric boundary of the disease-gene interval at D17S792. Recombination mapping thus defined a 16-cM interval, between D17S931 and D17S792, that must contain the multiple synostoses–syndrome gene.

Discussion

We found linkage of multiple synostoses syndrome to loci on chromosome 17q21-22. SYM1, a distinct but phenotypically similar autosomal dominant disorder, has also been localized to chromosome 17q21-22. The SYM1 interval was defined between the loci D17S579 and D17S795, an ~30-cM region. The 16-cM multiple synostoses–syndrome interval defined here is wholly contained within the SYM1 interval, providing evidence that they may be allelic disorders. Remaining recombinants, three on the centromeric side and one on the telomeric end, should allow the interval to be further narrowed as additional markers are defined.

Although a number of genes and ~140 expressed sequence tags have been localized to the multiple synostoses–syndrome interval, on the basis of tissue distribution, none of them appear to be good functional candidates. Some of the mapped genes in the region include the nucleolar transcription factor, cyclin A, and myeloperoxidase precursor genes, but these are general housekeeping genes that are not likely candidates for a disorder of cartilage. The HOXB gene cluster, a set of regulatory genes that specify anterior-posterior axis development, has also been localized to chromosome 17q21-22. Furthermore, mutations in a HOX gene from a different cluster, HOXD12 (Muragaki et al. 1996), cause limb malformations and have been identified in patients with synpolydactyly. Therefore, we considered the possibility that the HOXB cluster might harbor a disease-gene candidate. However, the primary site of expression of the HOXB genes is in the developing nervous system (Krumlauf 1994), and there is no evidence that the genes in the cluster, HOXB1–9, are expressed in cartilage. Furthermore, although the HOXB cluster is poorly mapped, the most likely location is in the D17S791–D17S797 interval (Human Transcript Map; Schuler et al. 1996), a region excluded by recombination mapping in our family. Thus, it is unlikely that mutations in one of the HOXB genes produces multiple synostoses syndrome.

Although geneticists have considered SYM1 and multiple synostoses syndrome to be genetically distinct disorders, the present linkage data suggest that they may be allelic. Clinically, two of the cardinal features seen in multiple synostoses syndrome—joint ankylosis and deafness—are seen in SYM1. Most interestingly, the pattern of expression of the symphalangism in both disorders follows a reproducible topographic course. It commences on the ulnar side and progresses radially, and it affects the proximal interphalangeal joints first and then advances to the distal joints. Thus, because the joint ankylosis extends beyond the phalanges, wrists, and ankles, multiple synostoses syndrome represents the more severe end of the clinical spectrum. In the family reported here, there are also significant cervical vertebral, radiohumeral, and hip-joint fusions. Whether this implies mutations in two tightly linked genes or different mutations in the same gene will be determined when the disease gene is isolated. Both of these disorders are progressive. From a mechanistic viewpoint, this implies an ongoing accumulation of an abnormal protein or, conversely, the absence of the function of a key gene involved in the maintenance of joint integrity. Because genes with primary responsibility for maintenance of the joint space have yet to be defined, isolation of the multiple synostoses–syndrome gene may provide new insights into one of the molecular mechanisms responsible for this process.

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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://genethon.fr/genethon_enhtml (for markers used for DNA typing)
Genome Database, http://www.gdb.org (for primer sequences used for DNA typing)
Human Transcript Map, http://www.ncbi.nlm.nih.gov/science96 (for the HOXB gene cluster)
International Skeletal Dysplasia Registry, http://www.csmc.edu/genetics/skeldys/default.html (for large family [reference R95-127] with multiple synostoses syndrome)
Online Mendelian inheritance in man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for multiple synostoses syndrome [MIM 186400/186500] and SYM1 [MIM 185800])
Whitehead Institute for Biomedical Research, http://www.genome.wi.mit.edu (for markers used for DNA typing)

References

Dib C, Faure S, Fizames C, Smason 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
Fuhrmann WG, Steffens CH, Rompe U (1966) Dominant erbliche doppelseitige Dysplasie und Synostose des Ellenbogengelenks mit symmetrischer Brachymesophalangie und Brachymetakarpie sowie Synostosen im Finger-, Hand- und Fusswurzelbereich. Humangenetik 3:64–75
Gaal SA, Doyle JR, Larsen IJ (1987) Symphalangism and its introduction into Hawaii: a pedigree. Hawaii Med J 46: 305–307
Herrmann J (1974) Symphalangism and brachydactyly syndrome: report of the WL symphalangism-brachydactyly syndrome: review of literature and classification. Birth Defects Orig Artic Ser 10(5):23–53
Hudson T, Stein L, Gerety S, Ma J, Castle A, Silva J, Slonim D, et al (1995) An STS-based map of the human genome. Science 270:1945–1954 (update: Whitehead Institute for Biomedical Research/MIT Center for Genome Research, Human Genetic Mapping Project, data release 12 [July 1997])
Hurvitz SA, Goodman RM, Hertz M, Katznelso MB-M, Sack Y (1985) The facio-audio-symphalangism syndrome: report of a case and review of the literature. Clin Genet 28:61–68
Krumlauf R (1994) Hox genes in vertebrate development. Cell 78:191–201
Lathrop GM, Lalouel JM (1984) Easy calculations of lod scores and genetic risks on small computers. Am J Hum Genet 36:460–465
Maroteaux P, Bouvet JP, Briard ML (1972) La maladie des synostoses multiples. Nouv Presse Med 1:3041–3047
Muragaki Y, Mundlos S, Upton J, Olsen BR (1996) Altered growth and branching patterns in synpolydactyly caused by mutations in HOXD13. Science 272:548–551
Murray JC, Buetow KH, Weber JL, Ludwigsen S, Scherpbier-Heddema T, Manion F, Quillen J, et al (1994) A comprehensive human linkage map with centimorgan density. Science 265:2049–2054
Polymeropoulos MH, Poush J, Rubenstein JR, Francomano CA (1995) Localization of the gene (SYM1) for proximal symphalangism to human chromosome 17q21-q22. Genomics 27:225–229
Schuler GD, Boguski MS, Stewart EA, Stein LD, Gyapay G, Rice K, White RE, et al (1996) A gene map of the human genome. Science 274:540–546
Strasburger AK, Hawkins MR, Eldridge R, Hargrave RL, McKusick VA (1965) Symphalangism: genetic and clinical aspects. Bull Johns Hopkins Hosp 117:108–127