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Report

The Gene for Juvenile Hyaline Fibromatosis Maps to Chromosome 4q21

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Juvenile hyaline fibromatosis (JHF) is an autosomal recessive condition characterized by multiple subcutaneous nodular tumors, gingival fibromatosis, flexion contractures of the joints, and an accumulation of hyaline in the dermis. We performed a genomewide linkage search in two families with JHF from the same region of the Indian state of Gujarat and identified a region of homozygosity on chromosome 4q21. Dense microsatellite analyses within this interval in five families with JHF who were from diverse origins demonstrate that all are compatible with linkage to chromosome 4q21 (multipoint LOD score 5.5). Meiotic recombinants place the gene for JHF within a 7-cM interval bounded by *D4S2393* and *D4S395*.

Juvenile hyaline fibromatosis (JHF [MIM 228600]) is an autosomal recessive condition that typically presents in the first few years of life with skin lesions, often on the scalp and ears, around the nose, and on the hands. These lesions may be papular and/or nodular, and the distribution and burden of lesions tends to increase throughout childhood, often necessitating recurrent excision. Gingival fibromatosis typically develops soon after the skin lesions and may also require excision. Progressive flexion contractures of joints increasingly limit movement and may result in individuals becoming wheelchair bound in early adulthood. Osteopenia is common; intelligence is normal. Diagnosis is confirmed by histological examination of the skin, which typically shows an abundance of a homogenous, amorphous, acidophilic extracellular hyaline material, in which spindle-shaped cells are embedded. The nature and origin of the hyaline material is unclear, but it appears to consist predominantly of collagens, glycosaminoglycans, and glycoproteins (Ishikawa et al. 1979; Mayer da Silva et al. 1988).

The condition has been termed "Murray syndrome," after the author of the original report (Murray 1873), and "Puretic syndrome," after the first author of a later case report (Puretic et al. 1962). It has also been called "juvenile systemic hyalinosis," but the modern term was introduced by Kitano et al. (1972). Predisposition to JHF is likely to be an autosomal recessive trait, because there is an equal sex distribution of cases; approximately one-third of affected children are siblings, and many are born to consanguineous parents. There is no apparent ethnic predilection, with ~40–50 cases from various populations reported in the literature (see Allen et al. 2001).

The underlying pathogenesis of JHF is unknown, and no consistent biochemical abnormalities are present. It has been postulated that JHF may be a disorder of collagen metabolism, because abnormalities of collagen III (Lubec et al. 1995; Breier et al. 1997) and collagen VI (Kayashima et al. 1994) have been reported in some patients. However, there are no consistent defects of collagen, and many affected individuals have normal col-

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lagen profiles. Some investigators have suggested that the abnormal collagen fibers result from an underlying defect in glycosaminoglycan formation (Remberger et al. 1985). However, although abnormalities in the biosynthesis of chondroitin sulphate and/or hyaluronic acid have been reported in some patients (Kitano et al. 1972; Iwata et al. 1980; Breier et al. 1997), they are not consistently observed.

We ascertained two families with JHF that both originate from a small village in a remote area of the Gujarat state of India (fig. 1). In family A, the proband (ID 7) presented at age 2 years with multiple subcutaneous swellings, initially on the scalp and later spreading to the face, extremities, and trunk. By age 10 years, she developed large nodules on the hands and feet that coincided with the underlying articular cartilage of the knuckles and phalanges (fig. 2). These nodules require surgical debulking at least twice a year. She has gingival fibromatosis, and, since the age of 20 years, she has been confined to a wheelchair because of progressive joint contractures of the ankles, knees, and hips. A radiological survey showed acro-osteolysis, and a skin biopsy revealed excess hyaline deposition. She has two affected distant relatives (ID 3 and ID 4), who have almost identical clinical histories, although their current mobility status is unknown. In family B, a single person (ID 9) is affected. He presented at age 2 years with skin nodules, originally around the nose and ears and subsequently on his fingers and toes. He also had severe gingival fibromatosis that required frequent debulking but became less prominent during his 20s. During childhood he developed progressive stiffness of the joints, predominantly the knees, shoulders, and elbows. A radiological survey showed generalized osteopenia, and his skin biopsy showed excessive hyaline deposition.

There is no known relationship between these two families, although both families are now living in Britain and have relatively little knowledge of their distant relatives. However, all ancestors of the affected children originate from the same relatively isolated region of India, where they lived for several generations. Given the rarity of JHF and the presumed autosomal recessive transmission, we hypothesized that all the cases in these two families were due to the same founder mutation and that the patients would be homozygous for identical alleles in the vicinity of the JHF-predisposition locus.

We performed a genomewide linkage search in four affected individuals, two unaffected siblings, and five parents from the two families, after informed consent was obtained. Four hundred microsatellite markers, spaced at 10-cM intervals, from ABI PRISM linkage-mapping set version 2 (Applied Biosystems) were amplified by PCR, using standard protocols. Amplified markers were electrophoresed on an ABI 3770 DNA capillary sequencer and were analyzed with GENESCAN and GENOTYPER software (Applied Biosystems).

JHF was modeled as a fully penetrant autosomal recessive trait, with a 0.01% population frequency of the disease allele and with equal recombination fractions in male and female individuals. Two-point LOD scores were calculated using Fastlink (Cottingham et al. 1993), and multipoint LOD scores were generated using GENEHUNTER 2.1 (Kruglyak et al. 1996). Marker-allele frequencies were estimated from unrelated individuals in the five pedigrees with JHF.

In the original genome scan, only one marker, *D4S2964,* was homozygous for the same allele in all four affected individuals. At *D4S392*—the marker centromeric to *D4S2964*—two of the affected individuals were homozygous for the same allele, and two affected individuals were heterozygous. At *D4S1534,* the marker telomeric to *D4S2964,* three affected individuals were homozygous for the same allele, and one individual was heterozygous. This suggested that the gene for JHF was between *D4S392* and *D4S1534* on chromosome 4q21. To evaluate this further, an additional 12 microsatellite markers from within the 17-cM interval flanked by *D4S392* and *D4S1534* were analyzed. The order and distance between these markers were obtained from the Marshfield Genetic Database, and they are, in order from centromeric to telomeric, *D4S392* (1 cM), *D4S2389* (2.8 cM), *D4S3042* (0 cM), *D4S1558* (0.7 cM), *D4S2915* (1.5 cM), *D4S2393* (2 cM), *D4S2963* (0 cM), *D4S2947* (1 cM), *D4S2964* (2 cM), *D4S2922* (0 cM), *D4S2932* (2 cM), *D4S395* (1 cM), *D4S1538* (1 cM), *D4S2361* (2 cM), and *D4S1534*. The four affected individuals from the two families were homozygous for identical alleles at all five markers between *D4S2393* and *D4S395*. None of the parents or unaffected siblings were homozygous through this interval (fig. 1*A* and 1*B*).

To further evaluate the JHF locus on chromosome 4q21, we ascertained and analyzed three additional families with JHF (families C–E [fig. 1]). Family C originates from Turkey and includes two affected sisters. Family D is from Morocco and includes two affected brothers. Both families are consanguineous. The clinical and histological findings in families C and D are classical for JHF and have been published elsewhere (Keser et al. 1999; Mancini et al. 1999). In family E, the proband (ID 26) was born to unrelated European parents and presented with erythematous-squamous lesions of the knuckles at age 1 mo (Rimbaud et al. 1974). At age 15 mo, a perianal tumor was removed. At age 18 mo, diffuse painful swelling of the joints and limitation of movements of large and small joints were noted. At 2 years of age, severe gingival fibromatosis was noted, together with nodules and papules around the lips, nose, scalp, and ears. Histologic examination of the skin lesions revealed fibrohyaline deposits, and a skeletal survey re-

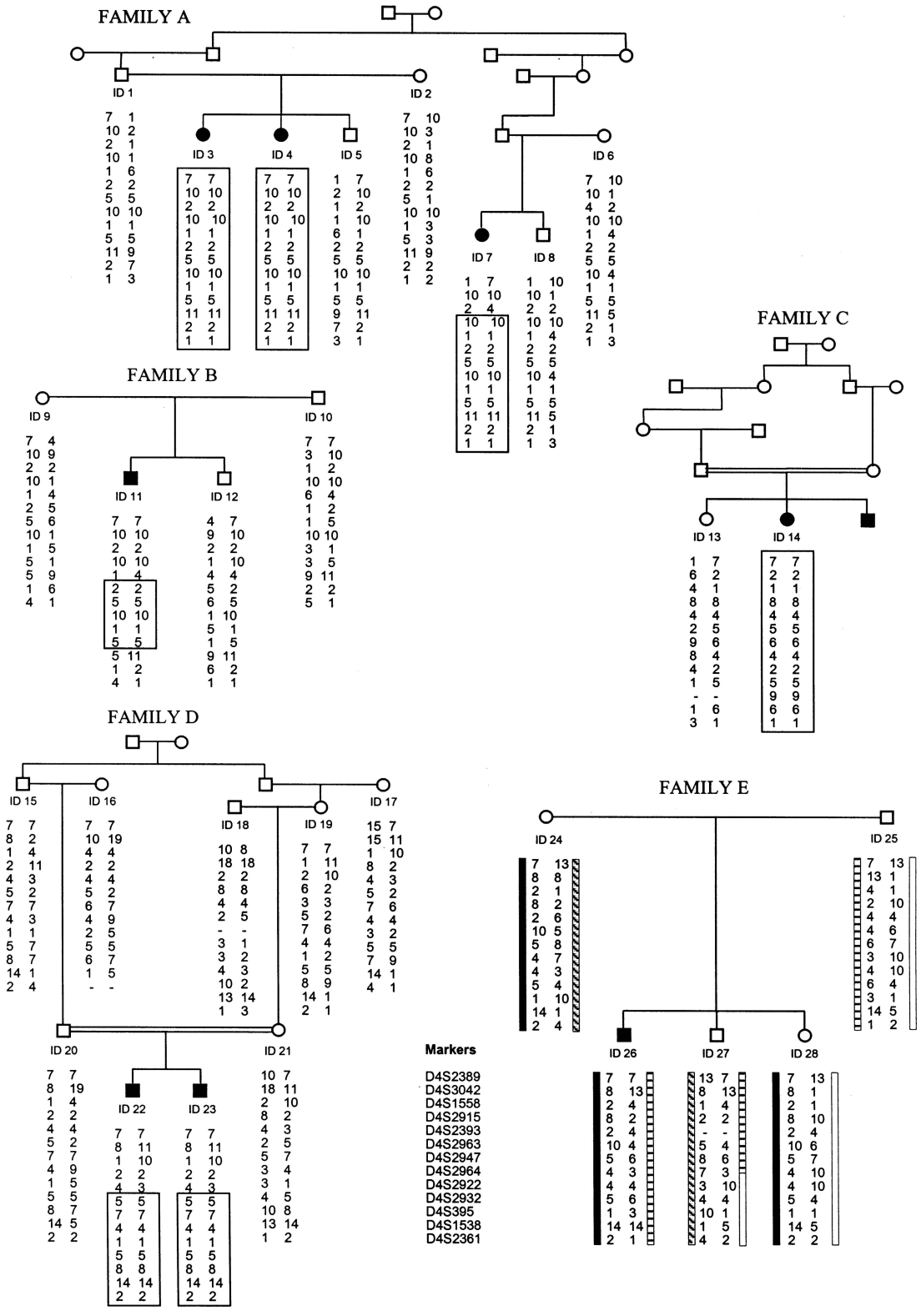

Figure 1 Haplotypes for 13 markers from chromosome 4q21 in five families with JHF. Blackened symbols indicate individuals affected with JHF. In families A–D, boxes around marker alleles indicate the region of homozygosity. In family E, all vertical bars next to the alleles indicate the segregating haplotypes and show that the parental chromosome 4q21 haplotypes inherited by the affected individual (ID 26) are different from those of his unaffected siblings. Critical meiotic recombinants in ID 11, ID 22, and ID 23 refine the minimal interval encompassing the JHF-predisposition gene to the region between *D4S2393* and *D4S395*.

vealed osteolytic lesions in the metaphyses. At age 20 years, the patient was still alive, but he had severe joint limitation.

Families C–E were analyzed with 13 microsatellite markers from the chromosome 4q21 interval that was defined in the original two families. In family C, the affected individual (ID 14) was homozygous at all 13 markers, whereas her unaffected sister was heterozygous through this interval. The marker alleles differed from those segregating in the two Gujarati families. This suggests that a separate Turkish founder mutation in the JHF gene is acting in family C. In family D, both affected boys are genotypically identical through the JHF region and are homozygous at all markers between *D4S2393* and *D4S2681*. Again, the alleles at these markers differ from those seen in both the Gujarati families and the Turkish family, suggesting that a separate mutation is responsible for the disease in these brothers from Morocco. In family E, the affected boy has inherited chromosome 4 haplotypes that are different from those of both of his unaffected siblings, consistent with mutations in the JHF gene on chromosome 4q21 acting in this family. The parents are not related, and the affected child has inherited different marker alleles from each parent and is thus likely to be a compound heterozygote for two separate mutations in the chromosome 4q21 JHF-predisposition gene, although it is possible that a different JHF-predisposition gene is acting in this family. The maximum two-point LOD score for all the families combined is 4.22 at *D4S395* (table 1). The maximum multipoint LOD score for all five families is 5.5 (fig. 3). In families B and D, critical meiotic recombinants that delimit the regions of homozygosity in these two families place the JHF-predisposition gene in a 7-cM region bounded by *D4S2393* and *D4S395*.

Table 1

Combined Two-Point LOD Scores at Chromosome 4q21 Markers in Five Families with JHF

	Two-Point LOD Score at θ =					
Marker	.00	.05	.1	.2	.3	.4
D4S2389	1.32	1.28	1.11	.72	.39	.15
D4S3042	2.28	1.87	1.60	1.12	.67	.30
D4S1558	1.76	1.40	1.19	.84	.52	.24
D4S2915	.94	.71	.57	.38	.24	.11
D4S2393	.90	1.11	1.04	.74	.44	.19
D4S2963	2.10	1.66	1.26	.64	.26	.07
D4S2947	2.34	1.92	1.54	.93	.52	.23
D4S2964	2.80	2.35	1.93	1.23	.71	.32
D4S2922	3.06	2.63	2.23	1.51	.91	.41
D4S2932	1.58	1.31	1.07	.68	.38	.16
D4S395	4.22	3.61	3.02	1.97	1.12	.47
D4S1538	3.90	3.33	2.79	1.81	1.02	.42
D4S2361	1.20	1.26	1.10	.70	.37	.13

Our data provide strong evidence for the location of a recessive JHF-predisposition gene on chromosome 4q21. The genomic sequence of the critical interval is incomplete, but scrutiny of the Project Ensembl and the University of California, Santa Cruz, Human Genome Project Working Draft databases indicates that the minimal interval is ~6 Mb and contains 19 known genes and 47 predicted genes. Several of the known genes are of potential interest as candidates, including bone morphogenetic protein 3 (*BMP3*), fibroblast growth factor

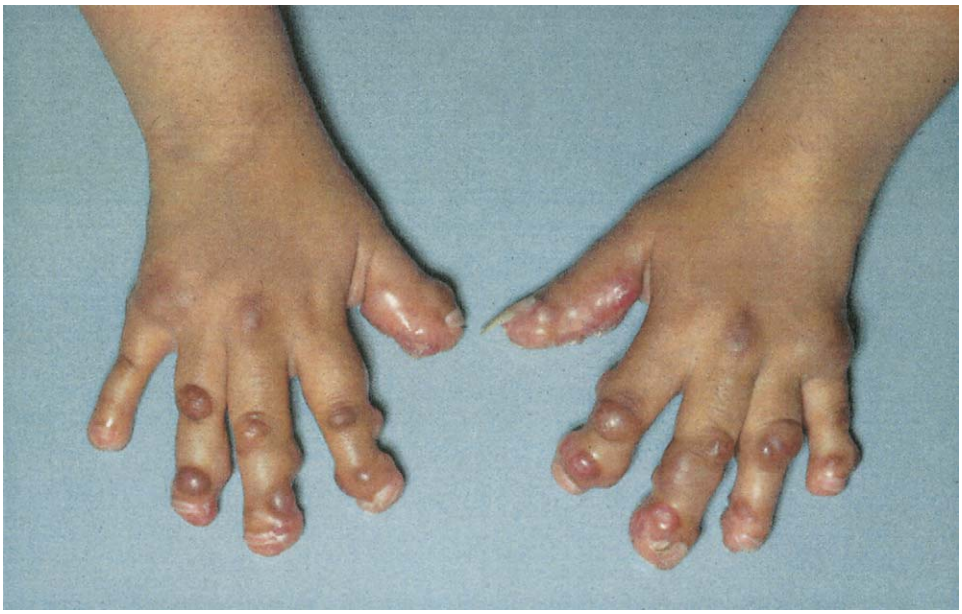

Figure 2 Hands of ID 7, showing nodular lesions and subcutaneous swellings that are typical of JHF

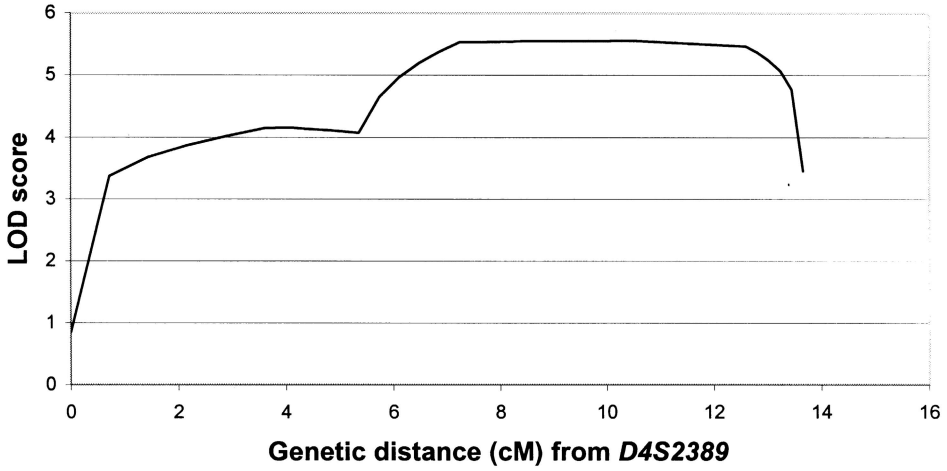

Figure 3 Combined multipoint LOD score at chromosome 4q21 in five families with JHF

5 (*FGF5*), protein kinase cGMP-dependent, type II (*PRKG2*), and the human homologue of mouse BMP-2–inducible kinase (*BIKE*).

Several conditions show some overlap with JHF, and they should now be examined for linkage to chromosome 4q21. Foremost among these is infantile systemic hyalinosis (ISH [MIM 236490]). This is an autosomal recessive condition that is also histologically characterized by hyaline deposits in the dermis. ISH has been primarily discriminated from JHF by its severe clinical course. Onset is in the first few months, typically with painful joint contractures and hyperpigmentation over bony prominences. Affected children develop early papules (predominantly on the face, scalp, and neck), fleshy nodules (particularly in the perianal region), and nodular plaques (particularly over the proximal interphalangeal joints of the hands). Gingival fibromatosis and osteopenia are often present. Affected children may have a protein-losing enteropathy and are susceptible to infections, and they usually die of overwhelming sepsis and/or multiorgan failure within the first few years of life. Autopsy reveals widespread hyaline deposition in many tissues, including skin, skeletal muscle, cardiac muscle, gastrointestinal tract, lymph nodes, spleen, thyroid, and adrenal glands (Landing et al. 1986; Glover et al. 1991, 1992; Sahn et al. 1994; Stucki et al. 2001).

Winchester syndrome (MIM 277950) also shows considerable overlap with JHF and ISH. This condition presents in early childhood and is characterized by short stature, coarse facial features, joint contractures, gingival fibromatosis, osteoporosis, and peripheral corneal opacities (Dunger et al. 1987; Prapanoch et al. 1992). Other conditions that share some features with JHF include congenital generalized fibromatosis (MIM 228550), the stiff skin syndrome (MIM 184900), lipoid proteinosis (MIM 247100), pseudo-Hurler polydystrophy (MIM 252600), Farber disease (MIM 228000), and I-cell disease (MIM 252500). However, these conditions should be distinguishable from JHF by clinical, genetic, biochemical, and/or histological findings.

It has been suggested that JHF may not be as rare as is generally assumed and that milder cases may be misdiagnosed because the histological appearances are not well known (Allen 2001). It is hoped that identification of the underlying predisposition gene will lead to development of a molecular diagnostic test and to better information about the incidence and phenotypic spectrum of the disorder. Advancement in our knowledge of the pathogenesis of JHF will allow insights to be gained into the fundamental defect in the condition and may also increase our understanding of the likely pathways and mechanisms that are subverted in conditions with related phenotypes.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation http://research.marshfieldclinic.org/genetics/ (for identification and order of microsatellite markers)
Ensembl, http://www.ensembl.org/ (for identification of candidate genes)
Online Mendelian Inheritance in Man (OMIM) http://www

.ncbi.nlm.nih.gov/Omim/ (for JHF [MIM 228600], ISH [MIM 236490], Winchester syndrome [MIM 277950], congenital generalized fibromatosis [MIM 228550], the stiff skin syndrome [MIM 184900], lipoid proteinosis [MIM 247100], pseudo-Hurler polydystrophy [MIM 252600], Farber disease [MIM 228000], and I-cell disease [MIM 252500])

UCSC Genome Bioinformatics, http://genome.cse.ucsc.edu/ (for identification of candidate genes)

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