

Report

A Genetic Locus for Adolescent Idiopathic Scoliosis Linked to Chromosome 19p13.3

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Adolescent idiopathic scoliosis (AIS) is one of the most common orthopedic disorders, affecting up to 4% of schoolchildren worldwide. We studied seven unrelated multiplex families of southern Chinese descent with AIS, consisting of 25 affected members. A genomewide scan with >400 fluorescent microsatellite markers was performed. Multipoint linkage analysis by GENEHUNTER revealed significant linkage of the abnormal phenotype to the distal short arm of chromosome 19, with both a maximum multipoint LOD score and a nonparametric LOD score of 4.93. Two-point linkage analysis by MLINK gave a LOD score of 3.63 (recombination fraction $\theta_{[m=f]} = 0.00$) at D19S216. Further high-density mapping and informative recombinations defined the AIS critical region in the vicinity of D19S216, flanked by D19S894 and D19S1034, spanning 5.2 cM on the sex-averaged genetic map on chromosome 19p13.3.

Scoliosis is the most common form of spinal deformity. It can be either idiopathic or secondary to congenital abnormality or to neuromuscular, connective tissue, or bone disorders (Lonstein 1995). Adolescent idiopathic scoliosis (AIS), which has no known etiology and occurs in otherwise healthy adolescents, accounts for ~80% of idiopathic scoliosis (IS [MIM 181800]). Its incidence among schoolchildren is 0.15%–4% (Lonstein 1995; Tse 1997; Reamy and Slakey 2001), with a third of cases (i.e., 5 per 10,000 schoolchildren) requiring immediate treatment with bracing or surgery (Lonstein 1995; Reamy and Slakey 2001). The identification of the disease gene or disease-susceptibility genes and the unraveling of the underlying defect may offer hopes for other therapeutic options.

AIS can be diagnosed clinically, by direct observation of the back or by the forward-bending Adams' test (Ad-

ams 1865). The extent of the curvature may be measured by calculation of the Cobb's angle, on radiographic evaluation (Cobb 1948). If left untreated, AIS can result in cosmetic problems and can cause lower back pain or deformity, and pulmonary function can be impaired in advanced cases, requiring mechanical ventilation. For the early and milder cases, bracing may prevent further progression during pubertal growth. For severe cases, surgical correction of their spinal curve, with its inherent risks (Newton et al. 1977; Luk et al. 1996), is the only choice. However, the correction rate after surgery is only 50%–60% (Liu and Huang 1996).

The development of scoliosis may be due to the relative difference in length or growth rate between the anterior and posterior portions of the spinal column (Murray and Bulstrode 1996). This may explain why adolescents with a particularly high growth rate during puberty are more prone to develop scoliosis. Familial occurrences of IS have been noted (Garland 1934; Wynne-Davis 1968; Riseborough and Wynne-Davis 1973; Harrington 1977), and concordance for this condition in MZ twins further strengthens the case for a hereditary basis of the disease (Kesling and Reinker 1997). However, controversy exists as to the mode of inheritance; on the basis of surveys mostly conducted by questionnaire and/or physical examination, it has been proposed to be a multifactorial or

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Table 1
Clinical Data of Proband of the Families with AIS

Family	Sex	Age at Presentation (years)	Location of Primary Curve ^a	Cobb's Angle at Diagnosis ^b	Spinal Surgery	Bracing
3	F	11	T6–T11	55°	Yes	No
8	F	13	T10–L3	26°	No	Yes
12	F	14	T3–T10	42°	Yes	No
13	M	11.5	T5–T12	42°	No	Yes
14	F	10	T5–T11	20° ^c	Yes	Yes
15	F	14	T9–L3	48°	Yes	No
18	F	10	T5–T11	22°	No	Yes

^a T = thoracic vertebra; L = lumbar vertebra.

^b A higher Cobb's angle value indicates a greater severity of AIS.

^c Curve continued to progress despite bracing, and surgery was performed when the Cobb's angle reached 50°.

complex trait (Fisher and De George 1967; Wynne-Davis 1968), an autosomal dominant trait (Garland 1934; Bell and Teebi 1995), or even an X-linked dominant trait (Cowell et al. 1972). With the advances that have been made in molecular genetics, it is possible to perform a genomewide search for the genetic locus/loci. In a study of four multiplex families with IS, Wise et al. (2000), using nonparametric linkage analysis, found evidence of allele sharing at three loci (chromosomes 6p, 10q, and 18q) in the affected individuals from two of these families. In the present article, we report our finding of a genetic locus responsible for AIS, mapped to chromosome 19p13 by linkage analysis.

We studied seven unrelated, multiplex families with AIS in Hong Kong, all originating from the Guangdong province of southern China. These families were identified through probands with severe AIS requiring surgery or bracing. All subjects, male and female, were ascertained by the same criteria. They were examined by the Adams' test and by a standing upright radiograph of the spine. The x-rays were read by two independent orthopedic surgeons who were blinded to the clinical state of the subjects. The Cobb's angle was measured, and AIS was defined as >10° lateral curvature of the spine. Individuals >12 years of age and having a normal Adams' test and x-ray results were considered to be unaffected. Unaffected individuals <12 years of age were classified as "phenotype unknown" for the purpose of linkage analysis. From the seven kindreds, 25 affected members (5 male and 20 female), 25 unaffected members, and 2 members with phenotype unknown were available for study. Among the affected members, six had received surgical correction of spinal curvature and three had bracing (table 1). All phenotypes were assigned prospectively before genotyping. Each participating subject—or, in the case of minors, the responsible adult—gave informed consent as approved by the Ethics Com-

mittee of the Faculty of Medicine at the University of Hong Kong.

Genomic DNA, extracted from peripheral blood leukocytes and Epstein-Barr virus-transformed lymphoblastoid cell lines, was used for genotyping. Four hundred fluorescein-labeled microsatellite markers distributed at ~10-cM intervals throughout the entire human genome (medium-density markers; ABI) were PCR amplified under conditions recommended by the manufacturer, and the products were analyzed on an automated sequencer (model 3700; ABI). Allele sizing and scoring were performed, with Genescan version 3.7 (ABI) software, by at least two persons unaware of the

Table 2
Two-Point LOD Scores for Linkage of Microsatellite Markers on Chromosome 19p13.3-13.2 to AIS at Different $\theta_{(m=f)}$ Values in All Seven Kindreds, under an Autosomal Dominant Model

MARKER	LOD SCORE ^a AT $\theta_{(m=f)}$ =				
	0	.1	.2	.3	.4
D19S209	-.888	.819	.782	.569	.295
D19S894	-1.179	1.822	1.561	1.052	.482
D19S216	<u>3.625</u>	2.934	2.136	1.301	.541
D19S1034	-.026	1.217	.918	.507	.148
D19S869	-.671	1.398	1.024	.557	.161
D19S406	-3.396	1.073	.892	.490	.135
D19S901	2.523	<u>3.512</u>	2.589	1.548	.613
D19S567	.546	2.201	1.677	1.035	.439
D19S873	-.752	.910	.702	.387	.113
D19S912	.491	1.825	1.349	.784	.294
D19S884	<u>4.020</u>	<u>3.184</u>	2.254	1.310	.495
D19S922	<u>4.087</u>	<u>3.150</u>	2.179	1.239	.450
D19S916	-2.287	2.137	1.672	.992	.378
D19S413	-1.607	.822	.692	.409	.127
D19S865	1.180	.876	.555	.270	.071
D19S914	-1.990	.015	.169	.135	.046
D19S221	-3.606	1.169	1.074	.715	.307

^a Significant LOD scores are underlined.

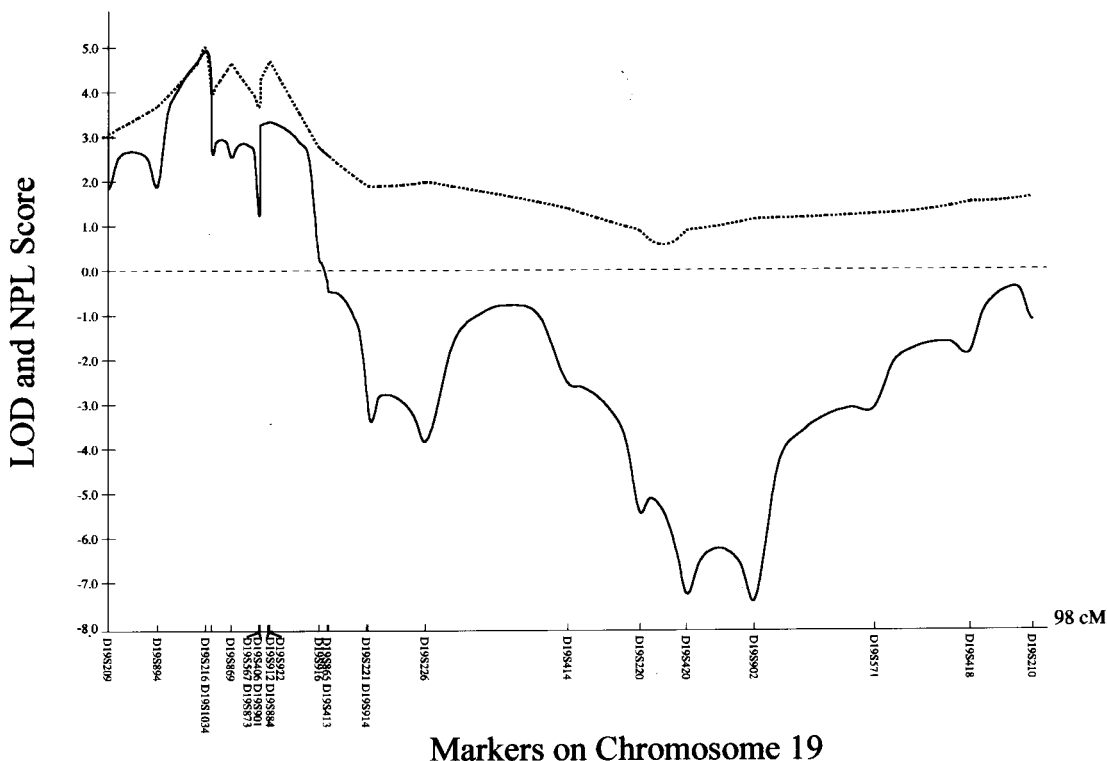


Figure 1 Multipoint linkage analysis of AIS to microsatellite markers on chromosome 19. The X-axis represents the relative location of these markers, and the Y-axis represents the multipoint LOD (solid line) and NPL (broken line) scores from parametric and nonparametric linkage analyses, respectively, for all seven kindreds.

subjects' clinical status at the time of scoring. Cyrillic version 2.1 (Cherwell Scientific) software was used for assignment of haplotypes.

Linkage analyses were performed, using parametric and nonparametric methods, by use of GENEHUNTER version 1.2 (Kruglyak et al. 1996). The allele frequency of each marker was calculated from our database or from married-in family members of the seven kindreds (Ott 1999). Two-point linkage analysis was performed with the MLINK software of the LINKAGE package version 5.2 (Lathrop et al. 1984). The map order and microsatellite marker locations were specified according to databases of the Center for Medical Genetics' Marshfield comprehensive human linkage map, the National Center for Biotechnology Information, the Whitehead Institute/MIT Center for Genome Research, the Genome Database, and the Stanford Human Genome Center Web sites.

Since Wise et al. (2000) found evidence of allele sharing at three genetic loci in affected members of two families with IS, we initially screened a multiplex three-generation family (family 3) through use of polymorphic markers on these chromosomal regions—6p, 10q, and 18q—but failed to confirm linkage when a unified multipoint link-

age approach was used (GENEHUNTER version 1.2). We next performed a genomewide scan for family 3 through use of 400 polymorphic microsatellite markers. Multipoint linkage analysis was used under a model of locus heterogeneity, specifying an autosomal dominant trait with a gene frequency of 0.01 and an estimated penetrance of 80%. Two intervals yielded initial multipoint LOD scores of >1. In particular, the locus at chromosome 19 yielded a multipoint LOD score of 1.75 and a nonparametric LOD (NPL) score of 6.71 ($P = .00293$). When we extended our study to six additional kindreds with AIS, multipoint linkage analysis of the seven families yielded a maximum LOD score of 4.48 and an NPL score of 5.36 ($P = .00003$) with markers on chromosome 19. We further genotyped 13 additional markers in the region flanked by D19S209 and D19S221, and the maximum multipoint LOD score and NPL score both were 4.93 at the region between D19S216 and D19S1034 (fig. 1). Two-point analysis with MLINK software yielded a maximum two-point LOD value of 3.63 at D19S216 (recombination fraction $\theta_{[m=f]} = 0.00$). Further computation using hypothetical sets of different marker allele frequencies was performed, to exclude the possibility of reporting false-positive linkage (Ott 1992; Freimer et al. 1993). It did

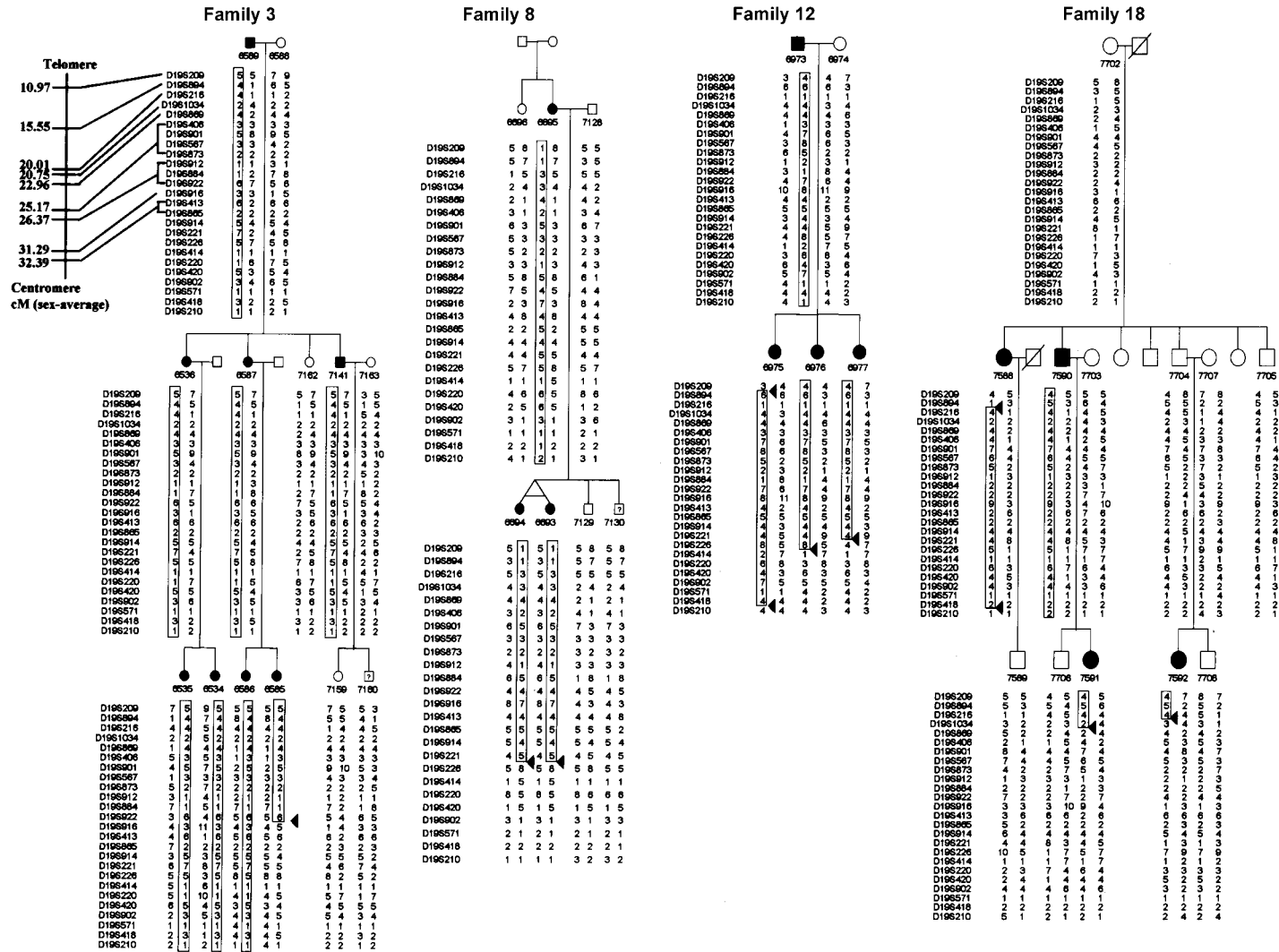


Figure 2 Abbreviated family pedigree and chromosome 19 haplotypes of four kindreds with AIS with informative recombinations. Affected individuals are denoted by blackened symbols, unaffected individuals are denoted by unblackened symbols, and individuals with unknown phenotype are indicated by a question mark. The identity of each microsatellite marker is shown to the left of each haplotype; their relative position along chromosome 19 is given in the top panel of family 3, and numbers on the far left are the sex-averaged chromosome distances (in cM) from the telomere of chromosome 19p. The individual number and haplotype is given under the symbol of each subject. The affected haplotype shared by relatives is boxed. An allele number represents a unique allele size across all families in the present study. Arrowheads indicate informative recombinations.

not significantly alter the multipoint LOD scores (which were 4.8–5.1) or two-point LOD values (which were 3.61–3.63 [$\theta_{m=f} = 0.00$]) at D19S216. Other loci, D19S884 and D19S922, gave two-point LOD values of 4.02 and 4.09 ($\theta_{m=f} = 0.00$), respectively (table 2).

A second chromosomal hotspot for AIS was located on chromosome 2, at D2S160-D2S347-D2S112-D2S151. A multipoint model-based linkage analysis showed a multipoint LOD score of 1.72, with an NPL score of 3.24 ($P = .00357$). No evidence of linkage was found with markers on the X chromosome.

Examination of the chromosome 19 haplotypes of all family members in the seven kindreds revealed several informative recombinations. Families 3, 8, and 12 defined an ~15-cM region for AIS (fig. 2). Examination of only affected members showed informative recombinations—for example, individual 6975 of family 12 defined the telomeric border at D19S209, and a recombination identified in individual 6585 of family 3 defined the centromeric border at D19S916. This finding was also supported by several recombinations identified in individuals of families 8 and 12.

Further detailed examination of the haplotype of family 18 shows a recombination in individual 7588 that defined the telomeric boundary at D19S894. Other recombinations in individuals 7591 and 7592 further narrowed the centromeric border to D19S1034. This encompasses a region of 5.2 cM on the sex-averaged genetic map.

Wise et al. (2000) reported positive NPL scores of 1.42, 1.60, and 8.26 at hotspots on chromosomes 6p, distal 10q, and 18q, respectively, in a single family. Only the 10q site was revealed in a second family (Wise et al. 2000). In the present study, we failed to confirm linkage to any sites in these chromosomal regions. The variation may be ascribed to the different phenotypes of scoliosis in the two studies, since we have only recruited patients in whom scoliosis developed during adolescence. It is also generally accepted that AIS is a complex disease in which one or more genes may be responsible for the expressed phenotype and in which other modifying effects, such as age, sex, and environment, may play specific roles in the phenotypic variation between affected individuals (Miller 2000). The reduced penetrance (80%) assumed in the present study is conducive if the prospective candidate gene in this region is a disease-susceptibility gene and if the expression of the disease phenotype is dependent on the interaction of environmental and other genetic factors. The significant and similar LOD values for both parametric and nonparametric analysis of the seven kindreds is an indication that our model of a dominant mode of inheritance and locus heterogeneity is correct.

Informative recombinations observed in four of the families effectively narrowed the AIS critical region to around D19S216, between D19S894 and D19S1034,

spanning 5.2 cM. This corresponds to a 1.9-Mbp region in the latest draft human genome sequence (Build 28). Within this region, there are ~71 possible gene sequences identified. Genes expressed in chondrocytes, osteoclasts, muscles, or tendon could be potential candidate genes for AIS. Among these, the leucine-rich α -2-glycoprotein gene, which is similar to chondroadherin, expressed in chondrocytes, as well as the SH₃-domain GRB2-like 1 gene, which is similar to endophilin 2, expressed in osteoclasts, are two strong candidates. Sequencing of these genes to identify the molecular defect, as well as demonstrating segregation of the defect in all affected members of a family, would be necessary to establish their candidacy.

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Electronic-Database Information

The accession number and URLs for data presented herein are as follows:

Center for Medical Genetics, <http://research.marshfieldclinic.org/genetics/> (for the Marshfield comprehensive human linkage map)
 Genome Database, The, <http://www.gdb.org/>
 National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/>
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for IS [MIM 181800])
 Stanford Human Genome Center, <http://shgc-www.stanford.edu/>
 Whitehead Institute/MIT Center for Genome Research, <http://www-genome.wi.mit.edu/>

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