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ORIGINAL INVESTIGATION

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Assignment of a locus for autosomal dominant idiopathic scoliosis (IS) to human chromosome 17p11

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Abstract Idiopathic scoliosis (IS) is a spine deformity of unknown etiology. Family studies have suggested that IS may be inherited as a mendelian autosomal dominant trait. We have performed linkage analysis on a three-generation IS Italian family. A positive LOD score value of 3.20 at θ=0.00 was detected with marker D17S799 after a genome-wide scanning. Analysis of six flanking microsatellites confirmed the linkage and haplotype inspection defined an interval of about 20 cM between D17S947 and D17S798. This is the first locus reported for IS. We scored genes mapping in this interval and studied the heparan sulfotransferase genes as candidates on the basis of their biochemical role. No causative mutation was detected in the affected patients.

Introduction

Idiopathic scoliosis (IS, MIM181800) is a structural fixed lateral curvature disclosing by upright spine roentgenograms at least a 10° curvature by the Cobb method (Weinstein 1994) with a rotatory component of the spine. The diagnosis is suggested by body asymmetries observed during clinical evaluation and confirmed by X-ray. IS develops before skeletal maturity; no definite cause has been established so far (Weinstein 1994). The whole spine as well as the iliac crests must be checked to assess skeletal maturity, and any curvature must be surveyed on the roentgenograms. Seventy to eighty percent of structural scoliosis is clinically classified as idiopathic (Weinstein 1994). IS frequency has been estimated in the range of 1.5–3% for curves larger than 10°, 0.3–0.5% for curves greater than 20°, and 0.2–0.3% for curves greater than 30° (Weinstein 1994). IS occurs in about 2–3% of adolescents, with a similar prevalence in males and females presenting with small-magnitude curves (10°). For curves greater than 30°, however, most cases are diagnosed in adolescent girls, with a sex ratio of 7–1. IS occurs either as an isolated defect or in association with other malformations. Several studies have documented familial clustering of IS, suggesting that genetic factors play a role (Risenborough and Wynne-Davies 1973; Wynne-Davies 1973). In addition, a significant higher concordance rate for IS has been found in monozygous twins (73%) compared to dizygotic twins (36%) (Kesling and Reinker 1997). Autosomal dominant, X-linked, and multifactorial patterns of inheritance have been reported (Lowe et al. 2000; Wynne-Davies 1968). Segregation analysis has suggested a single gene as a major determinant of IS (Axenovich et al. 1999). Different candidates, including COL1A1, COL1A2, COL2A1, and FBN1 genes have been examined by linkage studies, with negative results (Carr et al. 1992; Miller et al. 1996).

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Materials and methods

Family recruitment

We investigated a three-generation family of Italian ancestry that includes 11 members affected by IS (Fig. 1). Each family member who agreed to participate was clinically examined; no signs of mutisystem involvement were detected. Individuals were scored as affected if they had physical and radiographic evidence of IS (Table 1). The minimal angulation in affected family members was 10° curvature, and the maximum was 20°. Informed consent was obtained from all subjects.

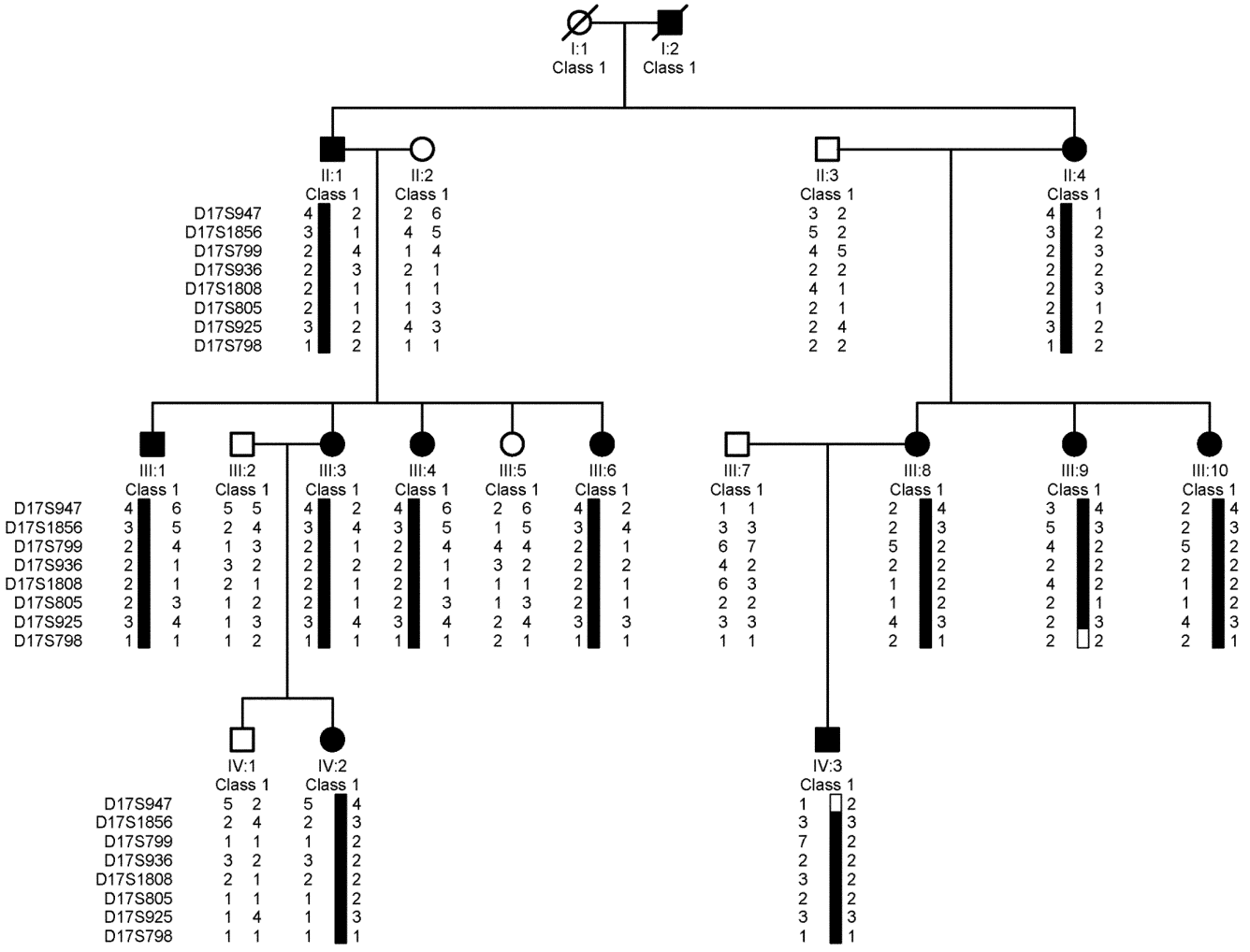

Fig. 1 Haplotypes of eight DNA markers on 17p11 in the examined family. *Open circles* and *squares* indicate unaffected members. *Black circles* and *squares* indicate affected members. *Black sections* of the *bars* indicate the haploidentical region in affected individuals, which defines the critical IS region between flanking markers D17S947 and D17S798

Table 1 [a]As stated by Weinstein (1994)

Subjects	Age (years)	Height	Body asymmetries	Idiopathic[a] scoliosis	Type of curvature	Vertebral rotation	Other skeletal deformity
II:1	68	178	+	+	Right thoraco–-lumbar	+1/2	–
II:2	64	165	–	–	–	–	Hypercyphosis
II:3	70	158	–	–	–	–	–
II:4	66	165	+	+	Right lumbar	+1	–
III:1	35	185	+	+	Right thoracic	+1/2	–
III:2	42	193	–	–	–	–	–
III:3	40	180	+	+	Right thoracic	+2/3	–
III:4	38	178	+	+	Double right thoracic left lumbar	+1	–
III:5	36	167	+	–	–	–	Hypercyphosis
III:6	31	176	+	+	Left thoraco–lumbar	+2	–
III:7	38	169	–	–	–	–	Hypercyphosis
III:8	35	162	+	+	Right thoracolumbar	+2	–
III:9	40	155	+	+	Right thoracolumbar	+1	–
III:10	32	165	+	+	Left lumbar	+1	–
IV:1	10	155	–	–	–	–	–
IV:2	12	158	+	+	Left thoraco–lumbar	+1	–
IV:3	9	140	+	+	Right thoraco–lumbar	+1	–

Genotyping

Genomic DNA was extracted from whole blood according to a standard protocol, quantified spectrophotometrically, and used at a concentration of 50 ng/µl. Genome scanning was performed with 358 microsatellite markers from the ABI prism linkage mapping set (PE Applied Biosystems). PCR was performed with 50 ng of DNA in a 15-µl reaction mixture containing 1.5 µl buffer (100 mM Tris HCl, pH 8.3, 500 mM KCl), 1.5 µl $MgCl_2$ (25 mM), 1.5 µl dNTPs mix (2.5 mM), 1 µl primer mix (5 µM), and 0.6 U of AmpliTaq Gold (PE Applied Biosystems). PCR products were analyzed on a 310 automated fluorescent DNA sequencer (PE Applied Biosystems) by Genescan collection software (version 3.1; PE Applied Biosystems). Data were analyzed by the Genescan analysis program (PE Applied Biosystems). Each marker was examined by the Genotyper program (version 2.0; PE Applied Biosystems).

Linkage analysis

Linkage analysis was performed with the Linkage 5.1 computer program package (Lathrop and Lalouel 1984) Two-point LOD scores between the disease gene and each marker were calculated using the MLink program (see Lathrop and Lalouel 1984; Mangino et al. 1999). The phenotype was coded as a fully penetrant autosomal dominant trait with a disease allele frequency of 0.0001. Equal recombination frequencies for men and women were assumed. The order of the markers and their recombination distances used for multipoint linkage analysis were based on the Généthon linkage map (Weissenbach et al. 1992; Gyapay et al. 1994; Dib et al. 1996). Multipoint analysis was performed by the Vitesse computer program (O'Connell and Weeks 1995).

Results

Clinical data

We studied a three-generation Italian family with eleven subjects affected by an autosomal dominant form of IS (Fig. 1). The whole family was clinically evaluated by X-ray, showing at least 10° curvature in the affected members (Fig. 2).

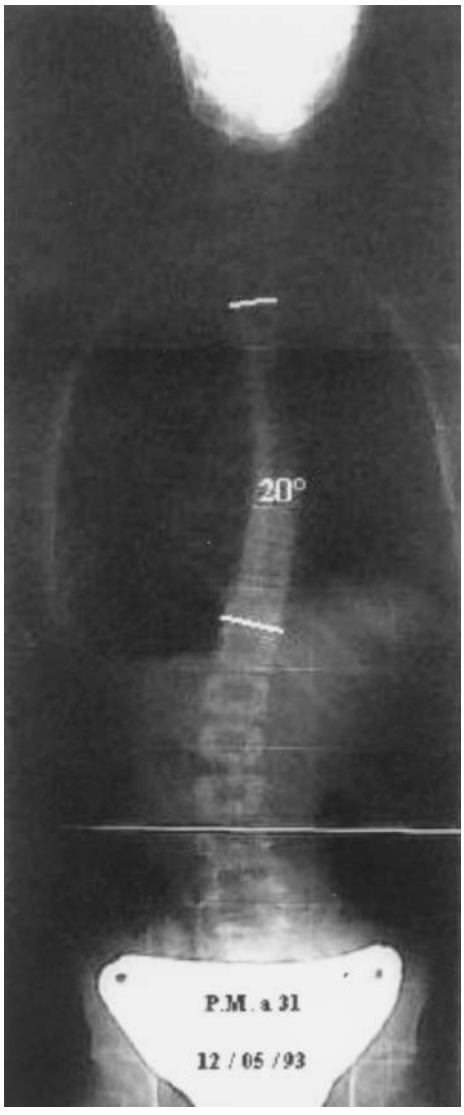

Fig. 2 Patient III:3, right thoracic thoracoscoliosis 20° Cobb, with left lumbar compensatory curvature

Linkage analysis

We performed a genome-wide scan with 358 fluorescent microsatellite markers selected from the ABI Prism linkage marker set (version 2, PE Biosystem, Palo Alto CA) which covers the entire human genome with a resolution of ~10 cM. A maximum two-point LOD score (Zmax= 3.20; θ=0.00) was obtained with marker D17S799 (Table 2). Haplotype analysis disclosed key recombination events between markers D17S947 and D17S799 in individual IV:3 (Fig. 1), defining the telomeric boundary of the disease locus (Fig. 1). The centromeric limit was determined by a crossover between markers D17S925 and D17S798 in subject III:9 (Fig. 1).

Markers D17S947 and D17S798 localized the IS gene to a ~20 cM region on chromosome 17p11. Multipoint analysis, performed by the Vitesse computer program (O'Connell and Weeks 1995) gave a maximum LOD score of 3.31, with a most likely location for the IS gene between markers D17S799 and D17S925. Many genes map in this disease region, including the heparan sulfate (glucosamine) 3-O-sulfotransferase 3A1 (HS3ST3A1 [MIM 604057]) and 3-O-sulfotransferase 3B1 (HS3ST3B1 [MIM 604058]) genes. Their protein products play a role in gly-

Table 2 Two-point LOD scores at eight polymorphic markers on chromosome 17p11

Markers	LOD score at θ=						
	0.00	0.01	0.03	0.05	0.10	0.20	0.30
D17S947	–∞	1.13	1.50	1.62	1.65	1.38	0.93
D17S1856	3.16	3.11	2.99	2.88	2.59	1.97	1.30
D17S799	3.20	3.14	3.03	2.92	2.62	1.99	1.30
D17S936	1.40	1.37	1.32	1.26	1.11	0.80	0.47
D17S1808	2.93	2.87	2.77	2.66	2.39	1.81	1.18
D17S805	3.18	3.13	3.02	2.90	2.61	1.98	1.30
D17S925	3.19	3.14	3.08	2.92	2.63	2.01	1.33
D17S798	–∞	0.62	1.01	1.14	1.22	1.04	0.70

coproteoglycan sulfatation, a key process for the organization of the osteo-ligamentous structures. Glycoproteoglycan abnormalities have been found in the Schwartz-Jampel syndrome (SJS [MIM 255800]), which is caused by mutations in the perlecan gene (HSPG2 [MIM 142461]), the major proteoglycan of basement membranes (Nicole et al. 2000). Intriguingly, the occurrence of scoliosis in SJS patients suggests a common pathogenetic mechanism mediated by proteoglycans. We therefore sequenced the coding regions of both HS3ST3A1 (GeneBank #NM_006042) and HS3ST3B1 (GeneBank #NM_006041) genes in two affected family members and one control individual. Three variations were identified in the HS3ST3A1 gene, unlikely to be pathogenic. The first is a three-base pair deletion (delGGA) in the transcription start upstream sequence, at position -1211-1209 from the start codon, also detected in normal controls. The second variation was a C to A transversion in the 5'UTR (-435C>A), and the third a silent C to T transition at codon 226 (g. 226C>T) in the second exon.

Discussion

The mapping of an IS locus on chromosome 17p11.2 is in agreement with some cytogenetic evidence relating this region to scoliosis. Imaizumi (Imaizumi et al. 1997) reported a patient, heterozygous for a de novo translocation t(13;17) (q34; p11.2), affected by congenital scoliosis. IS occurs in about 65% of Smith-Magenis syndrome patients (SMS [MIM 182290]), which are hemizygous for microdeletions of chromosome 17p11.2 (Smith et al. 1998). In addition, one third to one half of the patients with Charcot-Marie-Tooth type 1A (CMT1A [MIM 118220]) (Sturtz et al. 1997) – most of them resulting from duplication of the peripheral myelin protein 22 gene (PMP22 [MIM 601097]) that maps within the IS candidate region – are affected by spinal deformity with scoliosis. IS in CMT1A is not just of neuropathic origin, and the possibility that the duplication directly or by position effect involves other genes in the 17p11 region cannot be excluded. No study has yet reported the prevalence of scoliosis in CMT1A patients who have the duplication, as compared with the prevalence in patients with other mutations.

This study has provided the first evidence of a major autosomal dominant gene for IS, which is located on chromosome 17p11. The identification of this gene will lead to further insight into the process of skeletal development.

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