**Genomics Section, Inherited Disease Research Branch, NHGRI/NIH, Baltimore, MD**

**Department of Orthopaedic Surgery, Johns Hopkins University, Baltimore, MD**

**Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD**

---

**INTRODUCTION:**

Scoliosis is a structural abnormal curvature of the spine that occurs in otherwise healthy individuals during the late juvenile and early adolescence time period. Its prevalence among adolescents ages 10 to 16, is 25 per 1000. The high prevalence of this disorder, clinical variability, and evidence from population/family studies suggest that its expression is influenced by clinical and genetic heterogeneity. Through genome wide scans and statistical linkage analyses, familial idiopathic scoliosis has been linked to loci on chromosome 19p13.3 (7 Chinese families) [Chan et al., 2002], chromosome 17p11 (1 three generation Italian family) [Salehi et al., 2002], chromosomes 6p, distal 10q and 18q (1 two generation U.S family) [Wise et al., 2000]. Miller et al. [2005] in a large population of 202 families identified multiple primary and secondary regions of interest including chromosomes 1, 6, 7, 8, 9, 16 and 17 (202 U.S families). In this study, an independent sample of families using identical clinical criteria was utilized in an attempt to replicate the linkage findings reported by Miller et al. [2005].

**METHODS:**

**Ascertainment, Sample and Genotyping**

As part of a replication study of familial idiopathic scoliosis, 71 families (306 individuals) with at least two affected individuals (lateral curvature ≥ 10°) were ascertained and clinically characterized by a single orthopaedic surgeon (NHM). Measured and recorded variables included: the degree of lateral curvature, type of curve, age at diagnosis and type of treatment. One-hundred seventy-eight individuals were included: the degree of lateral curvature, type of curve, age at diagnosis and type of treatment. One-hundred seventy-eight individuals were determined to be affected. A genome-wide linkage screen with 385 autosomal STRP microsatellite markers was performed at the Center for Inherited Disease Research.

**Statistical Analyses**

Scoliosis was analyzed as a qualitative trait, with varying thresholds of curvature for affection status (10°, 21°, 30° and 40°) and as a quantitative trait measuring the degree of lateral curvature. Maximum likelihood estimates of founder-only allele frequencies for each of the 385 autosomal markers were obtained using the FREQ program [S.A.G.E. v.5.1, 2006]. The program GENIBD [S.A.G.E. v.4.5, 2001] was used to generate identity-by-descent (IBD) sharing distributions for full-sib pairs in the dataset via the exact multipoint algorithm without simulation. Single-point model-independent sib-pair linkage analysis was carried out with SIBPAL [S.A.G.E. v.4.5, 2001]. There were 239 sibships, ranging in size from 1 to 9 members, for a total of 401 sib pairs.

**Replication and Candidate Regions**

Regions with 2 or more markers significant at the 0.1 level in Miller et al. [2005] study were identified and combined with the p-value at the corresponding marker in this replication sample with Fisher’s combined probability test [Whitlock, 2005]. Replicated candidate regions were defined as markers for which the Fisher’s combined p-value was less than 0.01. Tightly linked flanking markers were included within the boundaries of candidate regions if they were marginally significant (Fisher’s combined p-value < 0.05).

**RESULTS:**

**Sample Population**

Characterization of families in the replication sample as compared to that of the original population resulted in similar distributions in relation to affection status, gender distribution of affected individuals, familial characteristics, curve patterns and type of treatment.

**Statistical Results**

A total of one-hundred twenty-six markers were identified at the 0.1 level in Miller et al. [2005] sample for at least one of the five phenotypic traits: scoliosis defined by thresholds at 10, 20, 30 and 40 degrees and the degree of lateral curvature. In the analyses of the replication sample, sixteen equivalent markers, located on chromosomes 1, 5, 6, 7, 9 and 15, had a p-value less than 0.1. (Results not shown.)

When analyzed by the Fisher’s combined test, nineteen markers had a Fisher’s combined p-value <0.1 across the five phenotypic traits. These markers combined with adjacent markers across traits resulted in eleven candidate regions located on 8 chromosomes (see Table 1). Eight of the eleven candidate regions correspond to those identified by Miller et al [2005] as primary or secondary candidate regions.

**DISCUSSION:**

The high prevalence and variability of scoliosis within the general population reflects the significant genetic and environmental interactions that may result in its expression. Strategies to minimize the effect of these influences and identify significant causative genetic factors include the identification of a homogenous population, and within that population to define genetically meaningful subgroups. The current work was successful in, first, the identification of a secondary independent population that was consistent in clinical parameters to an initial sample population. Secondly, through genome wide screening and statistical analyses, multiple candidate regions identified in the original sample were confirmed in the replication sample. This corroboration of critical genomic regions supports the conclusion that scoliosis is likely to be a genetically heterogeneous disease with several potential loci involved in its expression. Additionally, the replication of specific areas establishes these areas as essential target regions for further study.

**ACKNOWLEDGEMENTS**

Genotyping services were provided by the CIDR funded through a federal contract from the NIH to Johns Hopkins University (contract # N01-HG-65403). Some results were obtained with S.A.G.E., supported by the USPHS Resource grant 1 P41 RR03565. Additional funding has been provided by Johns Hopkins University, the Scoliosis Research Society, the Pediatric Orthopaedic Society, Cotrel Fondation, NIH grant ROI AR048862-03, and NIH/NHGRI, Division of Intramural Research, Inherited Disease Research Branch.