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CHD7 Gene Polymorphisms and Familial Idiopathic Scoliosis

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Abstract

Study Design—Model-independent linkage analysis and tests of association were performed for 22 single nucleotide polymorphisms (SNPs) in the *CHD7* gene in 244 families of European descent with familial idiopathic scoliosis (FIS).

Objective—To replicate an association between FIS and the *CHD7* gene on 8q12.2 in an independent sample of families of European descent.

Summary of Background Data—The *CHD7* gene on chromosome 8, responsible for the CHARGE syndrome, was previously associated with FIS in an independent study that included 52 families of European descent.

Methods—Model-independent linkage analysis and intra-familial tests of association were performed on the degree of lateral curvature considered as a qualitative trait (with thresholds of 10°, 15°, 20° and 30°) and as a quantitative trait (degree of lateral curvature). Results from the tests of associations from this study and the previous study were combined in a weighted meta-analysis.

Results—No significant results ($P < 0.01$) were found for linkage analysis or tests of association between genetic variants of the *CHD7* and FIS in this study sample, failing to replicate the findings from the previous study. Furthermore, no significant results ($P < 0.01$) were found from meta-analysis of the results from the tests of association from this sample and from the previous sample.

Conclusion—No association between the 22 genotyped SNPs in the *CHD7* gene and FIS within this study sample was found, failing to replicate the earlier findings. Further investigation of the *CHD7* gene and its potential association to FIS may be required.

Keywords

familial idiopathic scoliosis; genetic linkage; association analysis; meta-analysis; chromosome 8q12; CHARGE syndrome; CHD7

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Introduction

Late-onset idiopathic scoliosis (IS) is a structural lateral curvature of the spine greater than ten degrees that is noted in the prepubescent/adolescent period in otherwise normal children [1]. Clinical and genetic studies have shown that genetic factors influence the etiology of this disorder; however, the mode of inheritance is unclear [2–7]. Twin studies have resulted in a wide range of concordance estimates for IS, with rates ranging from 0.13 and 0.0 to 0.73 and 0.36 for monozygotic and dizygotic twins, respectively [8–10]. Autosomal dominant, X-linked dominant and autosomal recessive modes of inheritance have all been reported in the literature, suggesting that clinical and genetic heterogeneity may be involved in the expression of IS [2–7,11,12]. IS has been found to be significantly associated with single nucleotide polymorphisms (SNPs) in several genes in varying study populations including *MTNR1B* (melatonin receptor 1B) [13,14], *ESTR1* (estrogen receptor1) [15,16], and *MATN1* (cartilage matrix protein) [17]. An association between adolescent IS and a common variant near *LBX1* (ladybird homeobox 1) on 10q24.31 was identified in two independent Asian populations [18,19]. Based upon these findings, it is apparent that several genes may play a role in the manifestation of the disorder and that from family to family, the genes responsible may also vary.

The *CHD7* gene (chromosome domain helicase DNA binding protein 7) was reported to be associated with familial idiopathic scoliosis (FIS) in 52 families of European descent for which the proband had a curve of at least 15 degrees [20]. Mutations within the *CHD7* gene are responsible for the CHARGE syndrome (coloboma of the eye, heart defects, atresia of the nasal choanae, retardation of growth and/or development, genital and/or urinary abnormalities, and ear abnormalities and deafness) in which >60% of affected individuals who survive to adulthood have a growth disturbance of the spine that is similar to IS in its onset and phenotypic presentation [21]. The significance of this finding is that variants within the *CHD7* gene potentially underlie susceptibility of FIS, and maybe related to abnormal spinal growth and development. This study seeks to replicate the linkage and association findings with the *CHD7* gene in an independent population of families with FIS.

Materials and Methods

Sample Population and Genotyping

The study cohort was composed of 187 families (1021 individuals) with two or more individuals with a confirmed diagnosis of idiopathic scoliosis (IS, curvature $\geq 10^\circ$). Families were recruited with IRB approval for genetic studies of familial idiopathic scoliosis (FIS). Criteria for a diagnosis of scoliosis included history and physical examination consistent with a spinal curvature in the coronal plane, standing anteroposterior spinal radiographs exhibiting $\geq 10^\circ$ curvature by the Cobb method with pedicle rotation, and no congenital deformity [22–24]. Historical evidence or clinical signs including blood clots, cardiac defects, early onset osteoporosis, and hereditary disorders noted in any individual excluded the entire family from the study [25,26]. Additionally, individuals and families with secondary causes of idiopathic scoliosis were excluded from the study. The sample was consistent with previous epidemiological studies with respect to gender, curve type and curve size as measured by the Cobb angle [27].

In order to avoid misclassification, individuals without radiographic confirmation of disease status were classified as unknown (0.6%). All families were recruited in the United States and were of European descent. Of the 1021 individuals genotyped, 622 were classified as clinically affected, with curve severities ranging from 10° to 128° and averaging $31.67 (\pm 0.81)$, of which 76.9% were female.

Blood samples were obtained from all participants and genomic DNA was extracted using standard purification protocols [28,29]. The 23 single nucleotide polymorphisms (SNPs) in the *CHD7* gene that were analyzed in the previous study [20] were identified and genotyped in this familial study sample. SNPs were genotyped using the IlluminaBeadArray platform (Illumina Inc., San Diego CA). The IlluminaBeadStudio software was used to cluster all SNPs. SNPs were dropped if they had poorly defined clusters, excessive replicate or Mendelian errors, and/or more than 10% missing data. SNP rs13256023 was dropped due to poorly defined clusters.

Statistical Analyses

SNP positions were obtained from human genome build hg19 [National Center for Biotechnology Information (NCBI)]. Haploview was used to calculate Hardy-Weinberg equilibrium (HWE), minor allele frequencies (MAF), and linkage disequilibrium (LD) for all SNPs [30]. All SNPs were in HWE ($P > 1 \times 10^{-4}$).

Mendelian inconsistencies were checked with MARKERINFO (S.A.G.E. v6.0.1) [31]. Individuals with genotypes that were at least 10% missing or inconsistent were excluded from the analysis. Thirteen pairs of monozygotic twins existed in the dataset; one member of each twin pair was randomly dropped from the analysis. After data quality control measures, 184 families (992 individuals) remained in the analysis. For family-based association studies, families were retained for analysis if they consisted of at least one fully informative parent-offspring trio, which resulted in 148 families (858 individuals).

Spinal curvature measurements were treated as a quantitative variable, while the qualitative variable was defined by setting the affected threshold at lateral curvatures of 10° (Aff10), 15° (Aff15), 20° (Aff20), and 30° (Aff30). Allele frequencies and identity-by-descent values were calculated using FREQ and GENIBD [31]. Singlepoint and multipoint model-independent tests of linkage for the quantitative trait and the qualitative trait (Aff10, Aff15, Aff20 and Aff30) were performed using SIBPAL [31]. ASSOC was used to test for association with quantitative curve measurements [31]. Tests of association in the presence of linkage were performed using FBAT [32] for the Aff10, Aff15, Aff20 and Aff30 categories (Aff15 in Table 1; Aff10, Aff20, Aff30 data not shown). All p-values reported are nominal values.

A weighted meta-analysis was performed using Stouffer's Z-score [33] comparing the results of Gao et al. (TDTae, Table 1) [20] to the corresponding results of the intra-familial tests of association in this study for FIS with a threshold of 15° . A review of the literature (PubMed, Embase and Wanfang databases) did not identify any other articles published that evaluated the *CHD7* gene, associated polymorphisms and/or the identified locus on chromosome 8 that includes the *CHD7* gene within any IS study population.

Results

Table 1 includes the results of linkage and association analyses for an affected threshold of 15° , which is the threshold degree of curvature that Gao et al. used to define individuals as affected [20]. Of the 23 SNPs in the original panel, one SNP (rs13256023) did not meet quality control (QC) standards and was dropped. For the singlepoint linkage analyses, at an affected threshold of 15° , no SNP was significant at the 0.01 level, and only one SNP (rs4288413) was marginally significant ($P < 0.05$) (see Table 1). The only other instances where a nominal significance level of $P < 0.05$ was obtained was in a multipoint linkage analysis with an affected threshold 20° for rs4738813 ($P = 0.036413$, data not shown) and in a singlepoint linkage analysis with affected status defined at 10° for rs3763591 ($P = 0.027103$, data not shown). Linkage analyses (both singlepoint and multipoint) using degree

of lateral curvature as a quantitative measure did not result in any SNPs significant even at the $P < 0.05$ level.

Results from the association studies at the curve threshold of 15° from the current study, results from Gao et al. (TDTae) [20], and the results from the weighted meta-analysis are also presented in Table 1. No SNP was significant at the $P < 0.05$ level for the association analyses performed in our study, for qualitative trait with a 15 degree threshold (FBAT, Table 1) or for the degree of lateral curvature (data not shown). Furthermore, weighted meta-analysis of the two independent studies did not reveal any even marginally significant results ($P < 0.05$).

Discussion

Familial idiopathic scoliosis (FIS) is one of the most common inherited disorders of the immature skeleton, and is considered a complex genetic disorder, in which multiple genes potentially contribute to disease susceptibility, prognosis, and variability. The discovery of an associated gene or molecular pathway would provide insight into the underlying molecular mechanisms leading to spinal instability. Previous work identified an association between SNPs within the *CHD7* gene and FIS susceptibility in a sample population of families of European descent [20]. This study attempted to replicate these findings in an independent sample of FIS families with similar ethnicity. In this study, we identified three marginally significant results with linkage analysis. However, our results were not significant for tests of association to the *CHD7* gene within this FIS study sample. Furthermore, a meta-analysis of the two study samples indicated no association of the *CHD7* gene to the phenotype of FIS.

CHD7 is a member of the chromodomain helicase DNA-binding (CHD) protein family that plays a role in transcription regulation by chromatin remodeling. Mutations within the *CHD7* gene result in CHARGE syndrome (coloboma of the eye, heart defects, atresia of the choanae, retardation of growth and/or development, genital and/or urinary abnormalities, and ear abnormalities), an autosomal-dominant malformation syndrome in which 60% of individuals who live beyond early childhood will develop an idiopathic-like scoliosis [21]. A review of all *CHD7* variants note that 1) not all clinically diagnosed individuals with CHARGE syndrome have *CHD7* mutations; 2) *CHD7* mutations have also been identified in non-CHARGE syndrome patients, and, 3) benign *CHD7* variants have been identified in individuals who are phenotypically 'normal' [21,34].

Individuals with one feature of CHARGE syndrome (scoliosis, cleft lip/palette, congenital heart defects) have also been studied for *CHD7* variants with success limited to a study sample of families with FIS [20]. The hypothesis that minor variants of the *CHD7* gene may lead to or contribute to the idiopathic scoliosis phenotype is reasonable given the genetic variability and phenotypic features of the disease.

The high prevalence of FIS within the general population and its variability in expression is indicative of a significant degree of heterogeneity within any sample of affected families. In an effort to minimize effects of heterogeneity, the current work was directed to the identification of a sample of families with characteristics similar to those in the previously published study. The previous results, however, were not replicated in the current sample. The potential exists that the multifactorial nature of this complex disorder may deter the ability to decipher causative or contributory genetic variants across the two independent study samples given the subtle differences of sample identification and recruitment, the variable geographic areas, and the limited size of the study samples themselves.

Statistical meta-analysis has been applied to genomic studies in an effort to align resulting data from variable resources and study groups. Limitations of the application of current meta-analysis exist within this setting. The potential weaknesses and biases of genetic association studies, selection bias, and aspects of study design can affect meta-analysis results. Due to the significant difference in sample size within the two studies, a weighted meta-analysis approach was used to reduce potential bias that may exist between the two study samples because of their difference in size. Despite this strategy, no significant association was found between the *CHD7* polymorphisms and the phenotype of FIS.

Familial idiopathic scoliosis is a complex genetic disease in which multiple genetic variants and environmental influences may ultimately be responsible for its initiation and progression. The initial positive findings relating associations between *CHD7* gene variants to the scoliosis phenotype in a select familial sample could not be corroborated in a second independent sample with a similar ethnicity. It is possible that we failed to replicate the association between *CHD7* and FIS because of the amount of genetic heterogeneity within the FIS phenotype. The failure to replicate the initial results raises questions on the relevance of the *CHD7* gene in the etiology of FIS. Further investigation of the *CHD7* gene and the surrounding region is required in order to delineate the specific implications of these cumulative results in the context of the etiology of this complex disease.

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Key Points

- *CHD7* gene fine mapping of an independent sample of familial idiopathic scoliosis (FIS) families.
- No association between 22 SNPs in *CHD7* gene and the observed phenotype of familial idiopathic scoliosis in this study sample.
- Further investigation of the *CHD7* gene and the surrounding region is required in the context of the etiology of FIS.

Table 1

Linkage, association and meta-analysis results for *CHD7* SNPs

SNP	Chromosome Position (bp) ^a	Linkage analysis		Tests of association		Meta-analysis (Stouffer's Z-score)
		Singlepoint	Multipoint	FBAT ^b	TD-Tac (Gao et al., 2007)	
rs4738813	61595671	0.0630	0.2429	0.5142 (143)	0.009	0.2203
rs12544305	61597809	0.5161	0.1553	0.6969 (118)	0.002	0.2462
rs9643371	61614884	0.4475	0.1553	0.5691 (126)	0.0007	0.1459
rs1017861	61618713	0.2197	0.1553	0.7955 (140)	0.002	0.4117
rs13256023	61648551	NA	NA	NA	1	NA
rs4288413	61652144	0.0336	0.1553	0.6183 (148)	0.03	0.3684
rs7000766	61657833	0.5849	0.1554	0.6981 (120)	0.0005	0.2035
rs28710955	61662348	0.3339	0.1555	0.8432 (143)	0.0008	0.4499
rs1483207	61667280	0.2088	0.1555	0.7507 (118)	0.007	0.3559
rs1483208	61667898	0.3339	0.1555	0.8432 (143)	0.003	0.5052
rs1038351	61670308	0.5789	0.1556	0.7740 (122)	0.0002	0.2443
rs7843033	61672515	0.5546	0.1556	0.6054 (122)	0.0002	0.1274
rs7002806	61683192	0.4785	0.1557	0.4573 (119)	0.009	0.1483
rs7842389	61685194	0.5040	0.1558	0.5068 (120)	0.001	0.1131
rs7017676	61691360	0.4588	0.1559	0.5539 (124)	0.0003	0.1066
rs35168272	61695705	0.2915	0.1561	0.7203 (142)	0.0008	0.2975
rs4392940	61699931	0.4926	0.1563	0.5017 (119)	0.0003	0.0858
rs4237036	61701057	0.2915	0.1566	0.7203 (142)	0.002	0.3312
rs13280978	61704055	0.2699	0.1567	0.7203 (142)	0.004	0.3597
rs4301480	61709059	0.4665	0.1573	0.5107 (119)	0.003	0.1415
rs10957159	61712443	0.4052	0.1589	0.9016 (73)	1	1
rs10092214	61726879	0.0626	0.1601	0.4728 (146)	0.434	0.4523
rs3763591	61764389	0.0584	0.1738	0.9427 (137)	0.288	0.8994

^aChromosome positions are from human genome build hg19.

^bNumber of families analyzed per SNP in parentheses.