Original Article

Genetic markers for idiopathic scoliosis on chromosome 19p 13.3 among Saudi Arabian girls: A pilot study

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BACKGROUND AND OBJECTIVE: Genetic locus linked to chromosome 19p for Adolescent idiopathic scoliosis (AIS) has been described. This study was carried out with the aim to find any significant linkage or association between three microsatellite markers (D19S216, D19S894, and DS1034) of chromosome 19p13.3 in Saudi Arabian girls with AIS.
MATERIALS AND METHODS: In eleven unrelated Saudi Arabian girls who were treated for AIS with Cobb angle of ≥30 degrees and in 10 unrelated healthy individuals, linkage analysis was performed using parametric and nonparametric methods by use of GENEHUNTER version 2.1. Multipoint linkage analysis was used in specifying an autosomal dominant trait with a gene frequency of 0.01 and an estimated penetrance of 80% at the genotype and the allele level. Fisher's exact test was used in the analysis of contingency tables for the D19S216, D19S894, and DS1034 markers.
RESULTS: The analysis between the patient group and healthy girls showed that at genotypic level there was no significant association of the markers and scoliosis D19S216 (P = 0.21), D19S894 (P = 0.37), and DS1034 (P = 0.25). Whereas, at the allele level, there was statistically significant association between the marker DS1034 (P = 0.008) and no significant association with the other two markers D19S216 (P = 0.25) and D19S894 (P = 0.17).
CONCLUSIONS: Our study shows that at genotypic level none of the markers reported earlier were associated with scoliosis but at allele level, marker DS1034 was significantly associated with patients with AIS. This allele marker on chromosome 19p appears important in the etiology of AIS.

Key words: Adolescent idiopathic scoliosis, chromosome 19p 13.3, genetic markers

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Introduction

Scoliosis is one of the most common spinal deformities occurring in the 2 to 4% of the school-going children.[1] Adolescent Idiopathic scoliosis (AIS) occurs without any known cause in otherwise healthy children and no sparing of any ethnic group.[2] Girls are at more risk of progression by a ratio of 3.6: 1,[3] but why the boys should have less progression is still unclear.[4] The incidence of scoliosis in Saudi Arabia was reported to be in the range of 0.16 to 0.5%[5,6] and 59% were AIS.[7] McCarthy[8] suggested that complications of scoliosis can be prevented by early diagnosis and appropriate treatment, hence school screening was established so that scoliosis could be diagnosed easily to prevent complications of late presentation. The reported complications of scoliosis surgery are serious[9] and this could be avoided in majority of children with AIS, if early diagnosis and proper bracing is used. In conservative societies, girls often remain covered and early deformities of the spine are missed easily and most of the children end up being treated operatively.

At present, there is no clear consensus regarding the genetic influence on scoliosis, but reports indicates that AIS is linked to few chromosomes,[10-15] and most of the linkage was found to be in familial scoliosis. Recently, Heary and Madhavan[16] suggested that scoliosis could be inherited due to autosomal dominant, X-linked, or multigene influence. Chan *et al.*[17] found in Asian families of AIS a genetic locus linked to chromosome 19p13.3 and defined the AIS in the critical region in the vicinity

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of D119S216, D19S894, and D19S1034, and this was further confirmed by Alden *et al*.[18] They concluded the genetic locus on chromosome 19p 13.3 as potentially significant in the etiology of scoliosis. Most of the reported studies were done on familial scoliosis and little is known on AIS in unrelated patients. Hence, we have done this pilot study to find the association of three microsatellite markers of chromosome 19p 13.3 among ethnic Saudi Arabian patients.

Materials and Methods

The proposal to study the known genetic markers on chromosome 19p13 in Saudi Arabian AIS patients and healthy girls was submitted and approval was obtained from the Research and Ethical Committee of College of Medicine, University of Dammam and King Fahd Hospital of the University, AlKhobar. We studied 11 unrelated ethnic Saudi Arabian girls living in Eastern Province of Saudi Arabia with AIS with a Cobb angle of ≥30 degrees. Eleven girls had routine radiographs and bending films, magnetic resonance imaging of the spine, and pulmonary function tests. All patients were operated with posterior fusion and bone graft. Controls were randomly selected with no known history of scoliosis in the family and were clinically examined to rule out any scoliosis or spinal affection and were classified as "phenotype unknown" for linkage analysis. As all the patients and adults were ≤18 years of age, an informed consent was obtained from the patients and parents.

Genomic DNA was extracted from peripheral blood using a standard procedure. The microsatellite markers D19S216, D19S894, and D19S1034 were studied to find any significant linkage or association between three microsatellite markers of chromosome 19 between patients with AIS and healthy controls. The microsatellite markers were amplified by PCR (polymerase Chain Reaction) with one primer labeled with a fluorochrome. The fluorescent amplification products were then analyzed by capillary electrophoresis using a genetic analyzer 3130 from applied biosystem to determine the size of the amplified product by comparison with a size marker. Linkage analyses were performed, using parametric and nonparametric methods, by use of GENEHUNTER version 2.1.[19] Multipoint linkage analysis was used for specifying an autosomal dominant trait with a gene frequency of 0.01 and an estimated penetrance of 80%. These parameters were taken from Chan *et al*.[17] Fisher's exact test was used in the analysis of contingency tables for the D19S216, D19S894, and D19S1034 markers and a *P* value of <0.05 was considered as statistically significant.

Results

Eleven girls with AIS and ten healthy subjects were studied. The parents of the patients were unrelated and there was no history of AIS in either of the families. In the healthy subjects, there was consanguinity among the parents. The three microsatellite markers with allele ID and frequency studied on chromosome 19 are given in Table 1, the analysis at the genotypic level of the studied population is shown in Tables 2 and 3 shows the analysis at the Allele level. The analysis between the patient group and healthy girls showed that at genotypic level, there was no significant association of the markers and scoliosis; D19S216 ($P = 0.21$), D19S894 ($P = 0.37$), and D19S1034 ($P = 0.25$). However, at the allele level, there was statistically significant association between the marker D19S1034 ($P = 0.008$) and no significant association with the other two markers D19S216 ($P = 0.25$) and D19S894 ($P = 0.17$).

Linkage analysis of the parametric method showed no significant linkage between the markers and AIS using a model-based approach. Using a model-free

Table 1: Microsatellite markers of chromosome 19 that are used in this study.

D19S894		D19S216		D19S1034	
Allele ID	Frequency Size	Allele ID	Frequency Size	Allele ID	Frequency Size
133 1	0.02	179 1	0.02	219 1	0.14
142 2	0.06	181 2	0.14	223 2	0.03
145 3	0.28	183 3	0.05	227 3	0.39
147 4	0.06	187 4	0.39	231 4	0.28
153 5	0.02	189 5	0.23	233 5	0.02
156 6	0.02	191 6	0.02	235 6	0.11
158 7	0.02	193 7	0.16	239 7	0.03
160 8	0.06				
162 9	0.34				
165 10	0.09				
169 11	0.03				

For each marker, we assigned a unique ID to each occurring allele size. The given frequencies are calculated from subjects included in the study.

Table 2: Analysis at genotypic level

	D19S894 ($P = 0.37$)														
	1 10	2 9	3 10	3 11	3 3	3 9	3 8	4 4	4 7	4 9	5 9	6 9	8 9	9 10	9 9
Healthy	1	0	0	1	0	1	1	1	1	0	1	1	1	1	0
Patient	0	2	1	0	1	0	3	0	0	1	0	0	0	1	2
	D19S216 ($P = 0.21$)														
	1 5	2 2	2 4	2 5	2 6	2 7	3 4	3 7	4 4	4 5	4 7	5 7	7 7		
Healthy	1	0	1	2	1	1	1	0	1	1	0	1	0		
Patient	0	1	0	0	0	0	0	1	3	3	2	0	1		
	D19S1034 ($P = 0.25$)														
	1 3	1 4	1 6	2 3	2 6	3 3	3 4	3 7	4 4	4 6	6 6				
Healthy	0	1	1	1	1	1	1	0	0	3	1				
Patient	1	3	0	0	0	2	3	1	1	0	0				

Table 3: Analysis at allelic level

	D19S894 ($P = 0.17$)										
	1	2	3	4	5	6	7	8	9	10	11
Healthy	1	0	3	3	1	1	1	2	5	2	1
Patient	0	2	6	1	0	0	0	0	11	2	0
	D19S216 ($P = 0.25$)										
	1	2	3	4	5	6	7				
Healthy	1	5	1	5	5	1	2				
Patient	0	2	1	11	3	0	5				
	D19S1034 ($P = 0.008$)										
	1	2	3	4	6	7					
Healthy	2	2	4	5	7	0					
Patient	4	0	9	9	0	1					

analysis also, there was no statistical significance and all nonparametric LOD (logarithm (base 10) of odds) score was equal to 0.

Discussion

To our knowledge, this is the first report on genetic markers in AIS in unrelated patients in the Arab population. We found that at the allele level, there was statistically significant association between D19S1034 marker on chromosome 19p 13.3 among girls of ethnic Saudi Arabian descent who had IS. For other markers which were tested, D19S216 and D19S894, there was no significant difference at the genotypic or at the allele level. Our findings were in contrast to the reported study of Chan *et al.*[17] They found that in majority of the families with AIS who are studied, the critical region was close to D19S216, between D19S894 and D19S1034.

It is generally believed that there is a genetic influence in AIS. This lead to various studies in families in whom children were affected with AIS. It was found that AIS is more common in monozygotic twins as compared with dizygotic twins.[20-22] Further studies showed that there are possible linkages at IS1, IS2, and IS3, and it is believed that follows the Mendelian Inheritance in man.[23] Lowe *et al,*[24] Bell and Teebi[25] suggested that there was evidence that AIS is inherited as autosomal dominant inheritance with partial penetrance, but Justice *et al,*[26] indicated that there is a possibility of X-linked inheritance. Even after extensive work, it appears that the mode of inheritance is still incompletely understood. Most of the reported work relates to AIS present in generations. In our patients, meticulous history did not indicate that at least in the last four generations no member had suffered from AIS. Genetic studies in AIS was always being focused on the familial groups and at present, multiple genes are suspected of causing IS. Miller *et al.*[14] suggested that the area of future study for familial AIS is within the region of chromosome 6, 9, 16, and 17. AIS occurs in patients with no family history, such as in our patients, but the incidence of IS without family history is reported to be very low.[27] Contrary to this, in more than 428 patients over the last 20 years, the incidence of familial scoliosis was <1%. This indicates that the familial scoliosis is much lower than seen in other countries. In such a situation, one has to look for the genetic influence which could be of autosomal recessive mode of inheritance.

AIS is known to human race since long and we are

yet to find the exact gene or genes which cause the disease and the mode of inheritance. In our population where familial scoliosis is rare, we compared the patient population with the healthy controls. In our small group, we found that at the allele level, there was statistically significant association between D19S1034 marker on chromosome 19p 13.3. This could be a direction for a study involving large number of patients and healthy controls which could conclusively shed light on the D19S1034 marker on chromosome 19p.13.3, but as of now, we can conclude that the marker D19S1034 of chromosome 19p.13.3 is significantly associated with AIS.

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