

## Original Article

# The association study of MMP2 polymorphisms with susceptibility to adolescent idiopathic scoliosis in Han Chinese

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**Abstract:** Objective: Idiopathic scoliosis is the most common pediatric spinal deformity affecting 1% to 3% of the population, and adolescent idiopathic scoliosis (AIS) accounts for approximately 80% of these cases, however, the etiology and pathogenesis of AIS are still uncertain. The current study aims to identify the relationship between MMP2 and AIS predisposition, to investigate the major effect of MMP2 Gene in AIS patients, and to identify the relationship between the genotypes of the SNPs and the clinical phenotypes of AIS. Methods: 187 AIS patients and 184 healthy controls were enrolled into this case-control study. Two single nucleotide polymorphism (SNPs) candidates in MMP2 gene were selected by Haploview 4.0 software, case-control study was performed to determine the relationship between MMP2 gene and AIS predisposition. Case-only study was performed to determine the effects of these variants on the severity of the condition. Results: Both -735 C/T and -1306 C/T were found to be associated with AIS predisposition. The ORs were observed as 2.549 (95% CI 1.3519-3.8579, P=0.0046), and 1.923 (95% CI 1.2381-2.676, P=0.0157) for -735 C/T and -1306 C/T respectively. There was statistically significant difference between main curve, severity and genotype distributions of these two SNPs. Conclusion: Genetic variants of MMP2 gene are associated with AIS susceptibility and may play an important role in the development of AIS in the Han Chinese. Different clinical phenotype of AIS might be related to different SNP loci.

**Keywords:** Spinal deformity, pediatric, adolescent idiopathic scoliosis, MMP-2, SNP

## Introduction

Adolescent idiopathic scoliosis is a structural, tridimensional spinal deformity characterized by lateral curvature of the spine with Cobb angle (which is a measurement used for evaluation of curves in scoliosis [1, 2]) greater than 10°. It affects 2-3% of the adolescent populations [3].

Despite extensive study, the etiology and pathogenesis of AIS is still uncertain [4], several divergent hypotheses have been postulated to better define this etiology [5, 6]. Genetics, growth hormone secretion, connective tissue structure, muscle structure, vestibular dysfunction, melatonin deficiency, and platelet abnormalities are major areas of research [5, 6]. Adolescent idiopathic scoliosis mainly affects girls in number and severity, several familial surveys of idiopathic scoliosis provided strong

evidence that genetic factors have a role in this condition [7]. Single nucleotide polymorphisms (SNPs) in the genes for estrogen receptor a (ESR1) [8], estrogen receptor b (ESR2) [9], matrilin 1 (MATN1) [10], melatonin receptor 1B (MTNR1B) [11], tryptophan hydroxylase 1 (TPH1) [12], interleukin-6 (IL-6) and matrix metalloproteinase-3 (MMP-3) [13] have been reported to be associated with AIS predisposition. However, many scientists believe that there are still some more candidate genes need to be elucidated [14].

72 kDa type IV collagenase also known as matrix metalloproteinase-2 (MMP-2) and gelatinase A is an enzyme that in humans is encoded by the MMP2 gene. The MMP2 gene is located on chromosome 16 at position 12.2. Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix (ECM) in normal physiological process-

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**Table 1.** Age distribution and Sex proportion between case group and control group

Item	Case	Control group	P value
Femal/male	111/76	121/63	>0.05
Age at diagnosis (years)	15.48±2.47	N.A.	N.A.
Age range (years)	9-31	10-30	>0.05
Mean MCA <sup>a</sup> ± SD <sup>b</sup> (°)	48.24±12.11	N.A.	N.A.
MCA range (°)	31-127	N.A.	N.A.

<sup>a</sup>The maximum Cobb angle (MCA). <sup>b</sup>standard deviation (SD).

es, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis [15]. Most MMP's are secreted as inactive pro-proteins which are activated when cleaved by extracellular proteinases [16]. MMP2 was considered to be of particular importance to intervertebral discs homeostasis, and increased expression and activity of MMP-2 has been documented in disc tissue with degenerative lesions [17]. Based on that, we hypothesize that MMP2 may influence adolescent idiopathic scoliosis by regulating the matrix formation and degradation surrounding the spinal cord and disc.

Although MMP2 polymorphism was reported to related with incidence of intervertebral disc degeneration (IVD) [18], there is no much evidence to prove that MMP2 polymorphism has relationship with susceptibility of AIS. The one of major reasons is that there is no enough AIS patients world widely. Fortunately, China huge population provides convenience to study the possible relationship between MMP2 and AIS.

There are two important SNP sites located in the promoter region of MMP2, -735 C/T and -1306 C/T, both two sites could significantly influence the MMP2 activity [19] and associated with bone disease [20]. In order to determine whether polymorphisms of MMP2 are associated with a predisposition to AIS in Chinese Han population, we selected these two single nuclear polymorphism sites to conduct a case-control study involving 187 AIS patients and 184 control subjects.

### Subjects and methods

#### Ethics statement

The study has been approved by the Ethical Committee of the Tianjin Union Medicine

Center; written informed consent was obtained from all subjects or their parents in the case of children.

#### Subjects for the case-control study

A total of 187 patients diagnosed with adolescent idiopathic scoliosis and 184 healthy controls in the Tianjin Union Medicine Center of Tianjin city were enrolled in this study between October 2009 and February 2015. All the control subjects were frequency-matched to the cases on age ( $\pm 3$  years), gender and the Han nationality; curve pattern, Cobb angles of main curve of AIS patients were recorded (**Table 1**). The following candidates were excluded: those with a history of congenital anomalies, neuromuscular diseases, endocrine disorders, skeletal dysplasia or connective tissue disorders, or mental retardation or mental illness, as well as psychiatric patients on medication affecting bone metabolism. From the 146 patients in the scoliosis group, all patients have a Cobb's angle above 30 degrees with high risk as they required surgery.

#### Evaluation of scoliosis angle

Normal standing postero-anterior radiographs were taken for each AIS patient upon their first visit. Standard techniques for measuring Cobb's angle were used, and if more than one curve was discovered, the most severe curve was selected for measurement. A Cobb's angle of less than 10 degrees was considered normal.

#### Genotyping method

Genomic DNA from all the subjects was extracted from peripheral blood leukocytes using a DNA isolation kit following the manufacturer's instructions (Invitrogen, USA). The -735 C/T polymorphism of the MMP2 gene was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods. The specific primer for detecting C (-735) T polymorphism in the MMP-2 gene was 5'-ATAGGGTAAACCTCCCCACATT-3' (forward) and 5'-GGTAAAATGACCCTGAGACCTG-3' (reverse); for detecting -1306 C/T, the forward primer was: 5'GCCATTGTCAATGTTCCCTAAAA-CA3', the reverse primer was 5'TGACTTCTGAGCTGAGCCTGAA3'. All the primers were synthesized by Invitrogen (subgroup in Shanghai,

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**Table 2.** Association analysis of genotype distribution of -735 C/T and -1306 C/T of MMP2

SNP loci	Major/ minor allele	Case group			Control group			P value (P <sup>a</sup> <sub>adjust</sub> )		OR <sup>c</sup> (95% CI) <sup>d</sup>
		C/C	C/T	T/T	C/C	C/T	T/T	Genotype <sup>a</sup>	Allele <sup>b</sup>	
-735	C/T	158 (84.5%)	28 (14.97%)	1 (0.53%)	139 (75.55%)	38 (20.65%)	7 (3.80%)	0.027	0.01	1.887 (1.174-3.033)
-1306	C/T	112 (59.9%)	63 (33.7%)	12 (6.4%)	82 (44.6%)	81 (44.0%)	21 (11.4%)	0.009	0.003	1.656 (1.199-2.2887)

<sup>a</sup>P-values were calculated using the Cochran-Armitage trend test. <sup>b</sup>P-values were calculated using the  $\chi^2$  test. <sup>c</sup>Allelic odds ratio. <sup>d</sup>Confidence interval (CI). <sup>e</sup>P-values were adjusted using the Bonferroni method for multiple tests.

China). PCR was performed in a 25- $\mu$ l volume containing 0.1  $\mu$ g of DNA, 2.5  $\mu$ l of 10  $\times$  buffer, 1  $\mu$ l of 25 mM MgCl<sub>2</sub>, 0.5  $\mu$ l of 10 mM dNTP, 0.5  $\mu$ l of 20  $\mu$ M primers (forward and reverse), 0.5  $\mu$ l Taq DNA polymerase (Promega, 5 U/ $\mu$ l) and an appropriate volume of sterile water. The parameter for amplification of the MMP-2 gene was pre-denatured at 95°C for 5 min followed by 35 cycles of 94°C for 45 s, 62°C for 45 s and 72°C for 1 min and a final extension of 72°C for 10 min. PCR was performed with a ABI 2720 PCR instrument. PCR products were purified (Agarose Gel DNA Fragment Recovery Kit; Takara, Japan) and digested with the Hinf I restriction enzyme (Ferments, Glen Burnie, MD, USA) for 16 h at 37°C. The digested products were electrophoresed in 2% agarose gel. Parts of samples were for sequencing in order to validate the result of PCR-RFLP.

Positive and negative controls were used in each genotyping assay, and 5% of the samples were randomly selected and run in duplicates with 100% concordance. The results were reproducible with no discrepancy in genotyping.

### Statistical analysis

SNPs were tested for deviation from Hardy-Weinberg equilibrium (HWE) using a chi-square goodness-of-fit test. Differences between cases and controls with respect to allele frequencies were evaluated using the Chi-Square test using SAS 8.2. All the data of two SNPs with polymorphisms were analyzed by the association analysis based on alleles and phenotypes of the SNPs. Odds ratio (OR) and 95% confidence intervals (CI) were computed by the unconditional logistic regression to estimate the relative risk for the single locus genotypes by an online software- SNP stats.

## Results

### Epidemiologic data analysis

There were no significant difference of age distribution and sex proportion between case group and control group (P>0.05) (Table 1).

### Hardy-Weinberg equilibrium analysis of the genotype distributions between case group and control group

Hardy-Weinberg equilibrium analysis showed that there was no difference of the genotype distributions of all of 12 SNPs between AIS group and control group (data not shown).

### Case-control association analysis based on SNPs genotype and allele

The distributions of the alleles and genotypes for these two SNPs are given in Table 2. Among them, the genotype frequency distributions of these two SNPs were statistically different between case group and control group (P=0.027 and 0.009, respectively). Besides that, distributions of the allele frequency with respect to -735 C/T and -1306 C/T were statistically different between case group and control group (P=0.01 and 0.003, respectively). Further analysis revealed that both two SNPs were all associated with AIS predisposition, the ORs were observed as 1.887 (95% CI 1.174-3.033) and 1.656 (95% CI 1.199-2.2887) for -735 C/T and -1306 C/T respectively.

### Case-only study

#### Association analysis between SNPs and gender in case group

Case group was divided into two groups according to gender of patients. Differences with respect to distributions of genotypes for both SNPs were analyzed between these two groups. As we can see from the Table 3 that none of the SNPs were observed to have significant difference (P>0.05), and no association between the SNPs and gender was detected.

#### Genotype polymorphism of the SNPs - cobb angles of scoliosis

In this study, cobb angles of all the patients of case group were over 30°. We analyzed the association of the SNPs genotype polymor-

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**Table 3.** Association analysis between genotype distributions of the SNPs and sex in case group

SNP loci	Male			Female			P
	C/C	C/T	T/T	C/C	C/T	T/T	
-735	67	12	0	91	16	1	0.692
-1306	45	24	5	67	39	7	0.953

**Table 4.** Association between SNPs and Cobb angles of scoliosis

SNP loci	Genotype	Cobb angle	Number	P <sup>a</sup>
-735	C/C	52.3±12.9	158	0.6723
	C/T	52.5±13.7	28	
	T/T	54	1	
-1306	C/C	53.4±9.8	112	0.5876
	C/T	50.9±10.9	63	
	T/T	51.7±12.2	12	

<sup>a</sup>P-values were calculated using the Kruskal-Wallis test.

phism with Cobb angles of scoliosis. There was no statistical difference between main curve, severity and the genotype distributions of both two SNPs which were shown in **Table 4**.

### Association analysis between SNPs and the location of main curve in AIS

187 patients with AIS were divided into 3 groups including group A (thoracic curve, 119 cases), group B (thoracolumbar curve, 31 cases), group C (lumbar curve, 37 cases). The curve pattern or characteristics of thoracic curve, thoracolumbar curve, and lumbar curve were that their scoliosis apexes were located in the intervertebral disc space between T2 and T11-12, at T12-L1, and at L12-L4, respectively. The genotype distributions of the SNPs were compared among these three groups and between A, B and C, respectively. There was no statistical difference between the genotype distributions of both SNPs and location of main curve, which were shown in **Table 5**.

### Discussions

Adolescent idiopathic scoliosis is a three-dimensional deformity of the spine with lateral curvature combined with vertebral rotation, which can directly affect the patient's physical and mental health [21, 22]. However, the etiology of adolescent idiopathic scoliosis is still

unclear. Currently, AIS is thought to be multifactorial disorder, differing degrees of interaction between multiple factors depend on linear and summation causality [23, 24]. Although AIS has been linked to multifactorial causes, genetic factors are considered the most important cause [25]. Although there are no much relative reports about the MMP2 and AIS, the MMP2 play an important role in bone metabolism [26], intervertebral disc degenerations, and several other spinal and bone disease [27]. These clues give us more confidence to further explore role of MMP2 associated with AIS. Therefore, in the current study, we screened two important SNPs in MMP2 gene from 187 patients with AIS and 184 control cases, and conducted an association analysis between genotype polymorphisms of MMP2 and the different clinical phenotype of AIS. Finally, both two SNPs were identified to have possible association with predisposition of AIS.

SNPs can be divided into two categories: coding SNP, located at genomic coding region, and non-coding SNP, at non-coding region. Non-coding SNPs are distributed at intron, 5' untranslated region (UTR), 3'-UTR and other non-coding regions [28]. SNPs located at 5'-UTR may participate in the process of transcription and translation of the regulatory gene [29]; SNPs located at 3'-UTR could affect mRNA stability and translation procession [30]; and SNPs located at intron may influence the process of pre-mRNA splicing [31]. SNPs located at these regions may induce quantitative and qualitative alterations in protein expression, thus correlates with some disease, which are often referred to as functional polymorphism [9]. In the current study, the -735 and -1306 were located at promoter region, and previous study has demonstrated that both two SNPs can influence the MMP 2 protein expression [32].

Association between genetic marker and disease may result from the following two cases: (1) genetic marker correlates with the pathogenesis and pathology of the disease, (2) genetic marker has strong linkage disequilibrium with pathogenic sites [33, 34]. In this study, we found that the allele frequency and genotype distributions of both two SNPs were statistically different between case group and control group, and significantly increase the risk of AIS for C allele.

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**Table 5.** Genotype distributions among Thoracic curve group, Thoracolumbar curve group

SNP loci	Group A (119)			Group B (31)			Group C (37)			P
	C/C	C/T	T/T	C/C	C/T	T/T	C/C	C/T	T/T	
-735	98	20	1	26	5	0	34	3	0	0.1546
-1306	72	38	9	17	13	1	23	12	2	0.1862

Group A: thoracic curve, group B: thoracolumbar curve, group C: lumbar curve. P-values were calculated using the Cochran-Armitage trend test.

In the study of association between MMP2 polymorphism and location of primary curve for patients with AIS, the genotype distributions of all of two SNPs were not found to be different among group A (thoracic curve), group B (thoracolumbar curve), and group C (lumbar curve) ( $P > 0.05$ ). We also confirmed that there was no statistical difference between two SNPs of MMP2 gene and the main lateral Cobb angle, which suggest that MMP2 may play an important role in the stating progress of AIS.

As a multifactorial inheritance disease, one or more genotypes change will lead to different phenotypes. Age, gender, and environmental factors also contribute to the diversity of gene phenotypes. Certain non-genetic risk factors for AIS, such as osteopenia and late menarche have been reported. However, these data were not collected in the present study, which limited our ability to evaluate of gene-environment interaction [2]. In conclusion, candidate genes correlated with abnormalities will not be confirmed if the ranges of the gene phenotypes are not controlled.

In addition, when conducting association analysis, difference in allele frequency from systematic errors between case group and control group may result in false-positive results. Those results due to the mismatch in race and region, gender, and age structure between case group and control group only reflect the variation in evolution history, population migration, gender, and age structure between different populations [35-38]. This hierarchical phenomena existing in the population will cause a poor repeatability in the genetic study of complex diseases, leading to false-positive results. Although we screen the participants of AIS group and control group with strict inclusion criteria in this study, we still cannot ensure that there is no such interference of hierarchical factors. Consequently, more samples or nuclear family

are badly needed to confirm our conclusions.

Although many controversies and unanswered questions about AIS, the most difficult one is the aetiopathogenesis of AIS. We do not know if AIS is the result of one entity or more factors. The identification of aetiopathogenetic factors will enable improved prediction of progression and could aid in the development of more specific treatments. In our study, we found that Genetic variants of MMP2 gene are associated with AIS and may play an important role in the development of AIS. Although it's too early to draw a conclusion, it was suggested that MMP2 might play an important role in the development of AIS.

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### Disclosure of conflict of interest

None.

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