

Original Research Article

A Study of the Association of Polymorphism rs5860110 and its Protective Role against Ankylosing Spondylitis in a Chinese Population

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Abstract

Purpose: To investigate whether secreted phosphoprotein-1 (SPP1) promoter variants are associated with susceptibility to ankylosing spondylitis (AS) in the western China.

Methods: The rs5860110 variant of SPP1 promoter was genotyped using direct sequencing in 120 unrelated AS patients and 106 ethnically matched healthy controls. All the patients were human leukocyte antigen (HLA) -B27 positive. Frequencies of different genotypes and alleles were analyzed among the AS patients and the controls.

Results: In AS patient group, frequency of +254 SPP1 promoter genotype was 0.483 in base TG/TG homozygotes and 0.517 in TG-(deletion)/TG-(deletion) homozygotes, while the values for the control group were 0.311 and 0.689, respectively. However, TG-(deletion)/TG heterozygote was not detected in this study. The rs5860110 single nucleotide polymorphism (SNP) in SPP1 was significantly different when all AS patients were compared to controls ($p < 0.01$).

Conclusion: The rs5860110 SNP in SPP1 has a protective role against AS in the selected Chinese population.

Keywords: Polymorphism, Ankylosing spondylitis, Secreted phosphoprotein-1, Sequencing, Genotype, Alleles

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INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory disorder characterized by inflammation in the spine and sacroiliac joints causing initial bone and joint erosion and subsequent ankylosis [1]. Most patients develop first symptoms of AS younger than 30 years of age [2]. More recent studies have shown that structural damage at initial presentation is the best predictor of further damage [3–5].

Secreted phosphoprotein-1 (SPP1, located on 4q21-q25) is a secreted arginine-glycine-

aspartate (RGD)-containing phosphoprotein with cell-adhesive and chemotactic properties both *in vitro* and *in vivo* [6]. SPP1 mainly contributes to host defence, bone formation, and wound healing by stimulating macrophage migration as well as protecting against viral and bacterial infections through its pro-Th1 effect [7]. It has been shown that The SPP1 polymorphisms were associated with susceptibility to systemic lupus erythematosus (SLE) [8-10]. In addition, it was associated with many types of cancer, such as nasopharyngeal carcinoma [11], colorectal carcinoma [12], breast cancer [13], non-small-cell lung cancer [14], gastric cancer [15]. These

results indicate that *SPP1* can act as a diagnostic marker.

AS patients' disease severity is largely genetically determined [16]. However, there are no relative reports about the relationship between *SPP1* polymorphisms and risk of AS currently. Previous study has confirmed that *SPP1* is overexpressed and with higher levels in the AS patients compared with controls [17,18]. Therefore, the aim of the present study was to examine the polymorphism of *SPP1* rs5860110 for an association with AS in humans in an effort to determine whether there is any evidence that a genetic predisposition to altered *SPP1* expression might explain the overexpression seen in human AS patients.

EXPERIMENTAL

Subjects

From May 2010 to October 2012, 120 unrelated AS patients (78 men and 42 women, the average was 39.5 ± 10.3 years, range from 18 to 52 years. All the patients were HLA-B27 positive) and 106 normal control (71 men and 35 women, the average was 35.8 ± 11.5 years, range from 15 to 48 years), were recruited after giving written informed consent. This study was approved by Ethics Committee of Autonomous Region people's Hospital (approval ref no. 20100301) [19]. Three milliliters of fasting venous blood was collected from all subjects, and then the serum and blood clotting were separated. Genomic DNA was extracted from white blood cells via phenol and chloroform extraction ethanol precipitation essentially following standard protocols.

Analysis of polymorphisms in the *SPP1* regulatory region

The rs5860110 SNP was genotyped by direct sequencing of the sense and anti-sense strands following polymerase chain reaction (PCR) amplification of the promoter regulatory region -27 to +584. PCR was performed using 50 ng DNA as a template under the following conditions: 95 °C for 7 min, then 30 cycles of 94 °C for 50 s, an annealing temperature for 60 s, and 72 °C for 60 s, with a final extension at 72 °C for 10 min. The PCR products were subjected to cycle sequencing with the respective forward and reverse primer using an automated ABI 3100 DNA sequencer by GeneCore Bio Technologies (Shanghai China).

Statistical analysis

Statistical analysis was performed using SPSS 11.5 software. One-way ANOVA and t-test was used to compare mean differences for continuous variables. Allele frequency was determined via direct counting. The distribution of genotypes in the AS patients and control were obtained using a Chi-square test, and significance was defined as $p < 0.05$.

RESULTS

Gene deletion and polymorphism

Direct sequencing of DNA fragments from the promoter regulatory region -27 to +584 in AS patients and controls. In AS patients group, frequency of +254 *SPP1* promoter genotype was 0.483 in TG/TG homozygotes and 0.517 in TG-(deletion)/TG-(deletion) homozygotes, and the control group was 0.311 and 0.689, respectively, but the TG-(deletion)/TG heterozygote was not detected in this study (Figure 1).

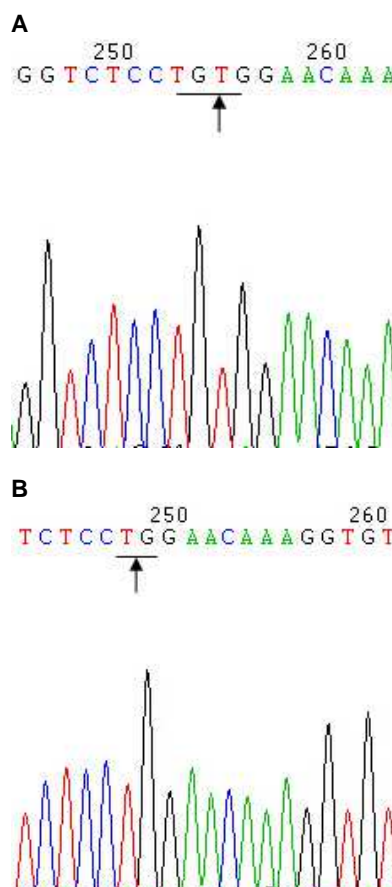


Figure 1: Schematic diagram and sequencing data of the *SPP1* promoter. **Note:** A = TG/TG homozygotes; B = TG-(deletion)/TG-(deletion) homozygotes

Table 1: Allele frequency of *SPP1* promoter polymorphism detected in AS patients and controls

SNP	Reference SNP	Allele	Frequency in AS patients	Frequency in controls	Chi-square	P-value
c.244_245ins TG	rs5860110	- TG	62(0.517) 58(0.483)	73(0.689) 33(0.311)	6.924	0.009

Compared with the controls, $p < 0.01$

***SPP1* promoter polymorphism is associated with AS susceptibility**

As shown in Table 1. The del TG frequency was significantly increased in controls (0.689) compared to AS patients (0.517) ($p < 0.01$), indicating that the haplotype del TG has a protective role against AS.

DISCUSSION

The pathogenesis of AS remains poorly understood. However, genetic factors play a significant role [20]. Single nucleotide polymorphisms (SNP) of the human *SPP1* gene has been reported to be associated with many diseases [21-23]. Based on my knowledge, it is first time to report the relationship between *SPP1* polymorphism and AS patients.

In this study, we have investigated the difference in *SPP1* gene polymorphisms between AS patients and healthy controls. In AS patients group, frequency of rs5860110 was 0.483 in TG/TG homozygotes and 0.517 in TG-(deletion)/TG-(deletion) homozygotes, and the control group was 0.311 and 0.689, respectively, but the TG-(deletion)/TG heterozygote was not detected. The del TG frequency was significantly increased in controls compared to AS patients ($p < 0.01$), indicating that the haplotype del TG has a protective role against AS. The studies about the rs5860110 SNP was relatively few. The rs5860110 frequency was 0.672 in TG-(deletion)/TG-(deletion) homozygotes in a German pseudoxanthoma elasticum (PXE) patients, and 0.801 in controls, the SNP could be interpreted as a genetic risk pattern for PXE [24]. A study found that The rs5860110 SNP was not associated with type 1 diabetic patients in Italy [25].

So far, genetic variants in the *SPP1* gene have shown to be involved in susceptibility to other immune-mediated diseases such as SLE [8,9], oligoarticular juvenile idiopathic arthritis [26] and sarcoidosis [27]. Despite promising functional data, previous genotype analyses could not confirm *SPP1* as significant disease modifying gene in classical Th17-mediated diseases such as multiple sclerosis [28,29] and rheumatoid

arthritis [30]. However, it is first time to report the relationship between *SPP1* polymorphism and AS patients. So the research should be confirmed in large and ethnically divergent population samples to make stronger conclusion about the association between the rs5860110 SNP with the AS.

CONCLUSION

This study demonstrates that genetic polymorphisms in the *SPP1* gene are associated with susceptibility to AS and that rs5860110 SNP has a protective role against ankylosing spondylitis in a Chinese population. This relationship is being reported for the first time.

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