Pakistan J. Zool., vol. 48(6), pp. 1753-1756, 2016.

A Single-Nucleotide Polymorphism in the Osteopontin Gene 9250 Contribute to Susceptibility to Ankylosing Spondylitis

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ABSTRACT

The aim of this study was to investigate possible association of a single nucleotide polymorphism (SNP) at position 9250 in exon 7 of the Osteopontin gene (OPN gene 9250) with ankylosing spondylitis (AS). A case–control association study was performed in 120 AS patients and 106 matched controls, consented to participate in the study. OPN gene 9250 polymorphism was detected by polymerase chain reaction (PCR) and direct sequencing. The frequency of the TC+CC genotype of the OPN gene 9250 was significantly higher (25.83% vs 12.26%, $p < 0.05$) and the frequency of C allele was significantly higher (17.50% vs 8.96%, $p < 0.01$) in AS patients than in controls. There were significant differences in OPN gene 9250 allele and phenotype frequencies between the AS patients and controls ($p < 0.05$). OPN gene 9250 polymorphism appears to be associated with susceptibility to AS in Chinese patients.

Article Information
Received 18 May 2015
Revised 10 February 2016
Accepted 12 February 2016
Available online 25 September 2016

Authors' Contribution
PT and QL conceived the study. PT and ZZ prepared, analyzed the data and wrote the article.

Key words
Ankylosing spondylitis, Osteopontin, single nucleotide polymorphism, Susceptibility

INTRODUCTION

Ankylosing spondylitis (AS) is an autoimmune-related disease characterized by chronic inflammations and gradual ossifications of the spine and sacroiliac joints (Brown *et al.*, 2000). The precise pathogenesis of AS is unknown, and a well-established genetic risk factor is associated with the HLA-B27 gene (Végvári *et al.*, 2009). More than 80% of patients are positive for the HLA-B27 allele, but only a minority of HLA-B27 carriers develop AS (1–5 %) (Cortes *et al.*, 2013). Therefore, other non-HLA-B27 variants are likely to influence susceptibility. Currently, 43 independent genetic loci have been shown to be associated with AS susceptibility (Cortes *et al.*, 2013).

Osteopontin (OPN) is a 44-kDa negatively-charged acidic hydrophilic, multifunctional protein encoded by the SPP1 gene that is located on chromosome 4q21-25 and consists of 7 exons (Sato *et al.*, 1999; Mazzali *et al.*, 2002; Forton *et al.*, 2002). OPN is mainly expressed in bone tissue, including osteoclasts as well as osteoblasts, and in other cell types, such as endothelial, smooth muscles, and epithelial cells (Mazzali *et al.*, 2002; Xie *et al.*, 2001). OPN is involved in the bone remodelling process (Chellaniah *et al.*, 2003; Dodds *et al.*, 1995). Bone cells secrete OPN physiologically during the process of bone remodelling. Osteoclasts may be the source of OPN in the cement lines of bone during remodelling (Chellaniah *et al.*, 2003; Dodds *et al.*, 1995). Therefore, OPN is an important modulator of stone formation. Mutations in the gene directing the synthesis of OPN may predispose to AS.

A single nucleotide polymorphism (SNP) at position 9250 in exon 7 of the OPN gene (OPN gene 9250) is detected in humans (Kikuchi *et al.*, 2003). The SNP was in the coding region of exon 7 of the OPN gene, thus the SNP at OPN gene 9250 might be associated with susceptibility to AS. AS patients' disease severity is largely genetically determined (Hamersma *et al.*, 2001; Wang and Cai., 2015). Previous study confirmed that OPN is thought to be a candidate molecule for the bone remodelling process in AS, in that it can induce both bone formation and resorption (Choi *et al.*, 2008). However, there is little report about the association of polymorphisms of OPN gene with AS to date. Therefore, we investigated the possible association of OPN 9250 polymorphism with AS of the patients in China, the OPN gene 9250 polymorphism has been detected in the patients and controls.

MATERIALS AND METHODS

Subjects

A total of 120 AS patients (male 78, female 42), and aged from 18 to 52 years (average 39.5±10.3 years), all the patients were HLA-B27 positive. And 106 controls (male 71, female 35) were recruited aged from 15 to 48 years (average 35.8±11.5 years). Consent for participating in the study was also obtained from them

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0030-9923/2016/0006-1753 $ 8.00/0

(Li *et al.*, 2014). This study was approved by the Ethics Committee of Autonomous Region People's Hospital (approval ref. no. 20100301) (World Medical Association, 2008).

Analysis of OPN gene polymorphisms

Blood samples of three milliliters were collected. DNA was extracted from peripheral blood leukocytes using TIANGEN Genomic DNA Purification Kit according to the manufacturer's instructions. SNP was determined using direct sequencing. PCR was performed using 50 ng DNA as a template under the following conditions: 94°C for 3 min, then 35 cycles of 94°C for 30 s, an annealing temperature for 45 s and 72°C for 30 s, with a final extension at 72°C for 10 min. The PCR products were genotyping by direct sequencing (ABI 3100 DNA sequencer by GeneCore Bio Technologies, Shanghai, China).

Statistical analysis

Differences in the frequencies of various alleles between patients with AS and control subjects were examined for statistical significance using the Chi-square test. One-way ANOVA and *t*-test were used to compare mean differences for continuous variables. Allele frequency was determined via direct counting, and A p-value less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

OPN genotype and allele frequencies

The OPN genotype was conducted using direct sequencing of DNA fragments from 9041 to 9292 in exon 7 of the OPN gene in AS patients and controls. The frequency of OPN gene 9250 with TT homozygotes in AS patients was 74.17% and 87.74% in the control (Fig.1).

Table I.- Allele frequency of *OPN* polymorphism detected in AS patients and controls.

	AS (n = 120)	Control (n = 106)	P-value
Allele, N (%)			
T	198(82.50)	193(91.04)	0.008[1]
C	42(17.50)	19(8.96)	
Genotype, n(%)			
TT	89(74.17)	93(87.74)	0.010[2]
TC	20(16.67)	7(6.60)	
CC	11(9.16)	6(5.66)	

[1]Compared with control, T vs C, $p < 0.01$; [2] Compared with the controls, CC+TC vs TT, $p < 0.05$

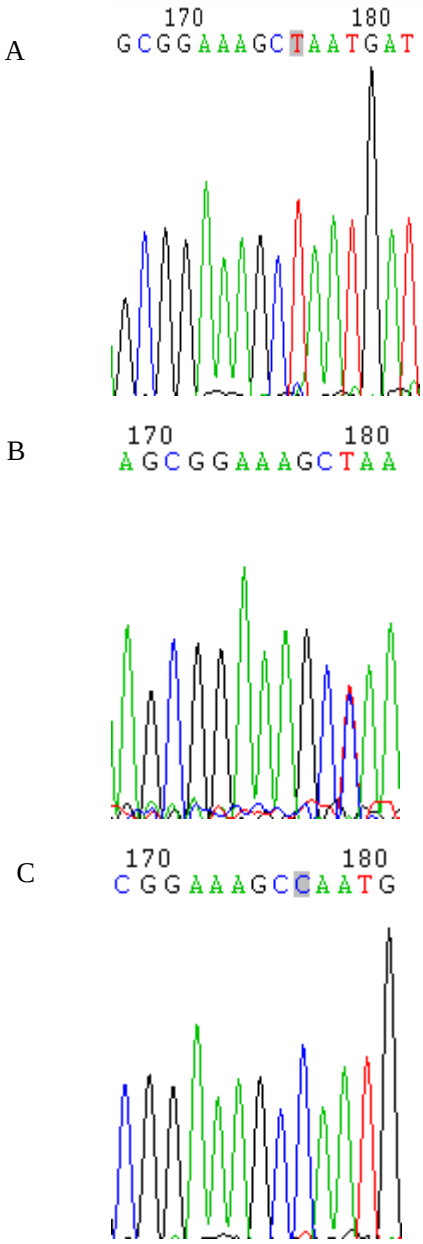

Fig. 1. Schematic diagram and sequencing data of OPN. A, T/T homozygotes; B, T/C Heterozygotes; C, C/C homozygotes.

OPN polymorphism is associated with AS susceptibility

As shown in Table I, C frequency was significantly increased in AS patients (17.50%) compared to the control (8.96%, $p<0.01$), the rate of the TC+CC genotype in AS patients was significantly higher than in those with CC genotype compared with control ($p<0.05$), indicating that subjects who carried C allele have a significantly higher risk of developing AS than in those with T allele.

DISCUSSION

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease that primarily involves the axial skeleton and the sacroiliac joint. It has long been known that susceptibility to AS is almost entirely attributable to genetic factors, and it is estimated that the heritability of the disease exceeds 90% (Reveille and Weisman, 2013). Previous studies have confirmed HLA-B27 as the major genetic key associated with AS (16-40%) (Peloso *et al.,* 2011). However, HLA-B27 cannot explain all patients with AS, as only 5% of HLA-B27-positive individuals develop AS (Duan *et al*., 2012), indicating that there are other contributing causes. It has been reported that AS is a complex genetic trait, with an estimated four to ten genes being responsible for most of the susceptibility (Cortes *et al*., 2013).

So far, the association of OPN gene polymorphism with AS is rarely reported. The data of our study showed that OPN gene 9250 polymorphism exists in the Chinese populations. The frequency of TT genotype of the OPN gene 9250 was significantly lower (74.17% vs 87.74%, $p < 0.05$) in the AS patients than in the controls, and the frequency of TC genotype of the OPN gene 9250 was significantly higher (16.67% vs 6.60%, $p < 0.05$). Significant differences were observed in the frequencies of OPN gene 9250 allele and phenotype between the AS patients and the controls ($p < 0.05$), indicating the association of OPN gene polymorphism with AS in Chinese populations.

Several polymorphisms have been described for the OPN gene, it has been shown to be involved in susceptibility to other immune-mediated diseases such as SLE (DAlfonso *et al*., 2005; Xu *et al*., 2007), oligoarticular juvenile idiopathic arthritis (Marciano *et al*., 2006) and sarcoidosis (Maver *et al*., 2009). A recent study reported that patients with the polymorphisms of the OPN gene in positions 8090 T/T + 9250 C/C, 8090 C/C + 9250 C/T, and 8090 C/T + 9250 C/T were linked with higher levels of disability in multiple sclerosis patients (Biernacka-Lukanty *et al*., 2015). Another study reported that OPN gene 9250 polymorphism appears to be associated with susceptibility to SLE in Chinese Han ethnic population (Xu *et al*., 2007).

However, it is first time to report the relationship between OPN gene 9250 polymorphism and AS patients, who carry C allele may have a significantly higher risk of developing AS. It is expected that this study should be confirmed in a large and ethnically divergent population in order to make a stronger conclusion about the association between OPN gene 9250 polymorphism with AS.

Limitations of the study

In this study, the sample size of participants was small, the result may be biased, large sample study need to be explored further.

ACKNOWLEDGMENTS

We wish to thank all the volunteers that participated in this study, We thank Tao Bao, Wuhan University, for expert technical help.

Conflict of interest

The authors declare that there is no conflict of interest.

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