

Original Research Article

Genetic Polymorphisms of Osteopontin in Association with Ankylosing Spondylitis in a Chinese Population

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Received: 7 August 2015

Revised accepted: 5 November 2015

Abstract

Purpose: To determine the association of osteopontin (OPN) polymorphisms with ankylosing spondylitis (AS).

Methods: A total of 120 cases diagnosed with AS and 106 age- and sex-matched healthy controls were recruited. All the patients were human leukocyte antigen (HLA)-B27 positive. Three single nucleotide polymorphisms were genotyped using direct sequencing

Results: The T allele at -443 SNP had significantly higher frequency in AS patients (0.1875) than the controls (0.1085, $p < 0.01$). The rate of CT+TT genotype in AS patients was significantly higher than those with CC genotype compared with the control ($p < 0.01$).

Conclusion: SNP at -443 of OPN gene can serve as a candidate genetic marker to evaluate the risk of AS, thus indicating that subjects who carry T allele have a significantly higher risk of developing AS.

Keywords: Genetic polymorphism, Ankylosing spondylitis, Osteopontin, Sequencing

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

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].

Osteopontin (OPN) is well known as a major non-collagenous protein related to bone remodeling [6-7]. The expression of OPN in different cell types was significantly influenced by transcription factors, and the genetic polymorphisms of the promoter [8]. Previous studies showed that OPN inhibited nucleation,

growth, and aggregation of calcium oxalate crystals *in vitro* [9,10], and was able to directly inhibit the binding of calcium oxalate crystals to cultured renal epithelial cells [11]. Therefore, OPN is an important modulator of stone formation. Mutations in the gene directing the synthesis of OPN may predispose to AS.

Recently, -443T/C polymorphisms on the promoter region of OPN gene have been found to affect gene expression and transcriptional activity [12,13], and have been found to associate with several diseases, including pseudoxanthoma elasticum [14], stroke [15], and chronic hepatitis C [16]. The -443 T/C polymorphism influenced the binding of MYT1 zinc finger factor, which is associated with neurogenesis [17]. In chronic hepatitis C

patients, the -443T/C polymorphism could be a marker reflecting hepatitis activity [18].

AS patients' disease severity is largely genetically determined [19]. However, there are no relative reports about the relationship between OPN polymorphisms and risk of AS currently. Previous study confirmed that OPN is overexpressed and with higher levels in AS patients compared with controls [20,21]. However, there have been no studies investigated the association of polymorphisms of OPN gene with AS to date. Therefore, we conducted a hospital-based case-control study to investigate the association of polymorphisms of OPN gene with the risk of AS in a Chinese population.

EXPERIMENTAL

Subjects

A case-control study was conducted between May 2010 and October 2012. A total of 120 unrelated AS patients included 78 men and 42 women with mean age of 39.5 ± 10.3 years (range 18-52 years, all the patients were HLA-B27 positive) and 106 normal control included 71 men and 35 women with mean age of 35.8 ± 11.5 years (range 15 - 48 years). All the subjects were recruited after giving written informed consent. This study was approved by Ethics Committee of Autonomous Region People's Hospital (approval ref. no. 20100301) [22].

Analysis of OPN gene polymorphisms

Three milliliters of fasting venous blood were collected from all subjects. Genomic DNA was extracted from the white blood cells following standard protocols. SNP was determined using direct sequencing of the sense and anti-sense strands following polymerase chain reaction (PCR) amplification of the promoter regulatory region -532 to +94. PCR was performed using 50 ng DNA as a template under the following

conditions: 94 °C for 7 min, then 30 cycles of 94 °C for 55 s, an annealing temperature for 50 s and 72 °C for 90 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 were used to compare mean differences for continuous variables. Allele frequency was determined via direct counting. Statistical significance of differences in gene and genotype frequencies between patients and controls was evaluated for each polymorphism using Chi-square test with Yates' correction, and significance was defined as $p < 0.05$.

RESULTS

OPN genotype and allele frequencies

The OPN genotype was conducted using direct sequencing of DNA fragments from the promoter regulatory region -532 to +94 in AS patients and controls. The frequency of SNP at -443 with CC homozygotes in AS patients was 0.7083 and 0.8396 in the control (Figure 1).

OPN polymorphism is associated with AS susceptibility

As shown in Table 1, T frequency was significantly increased in AS patients (18.75) compared to the control (10.85, $p < 0.05$), the rate of the CT+TT genotype in AS patients was significantly higher than in those with CC genotype compared with control ($p < 0.05$), indicating that subjects who carried T allele have a significantly higher risk of developing AS than in those with C allele.

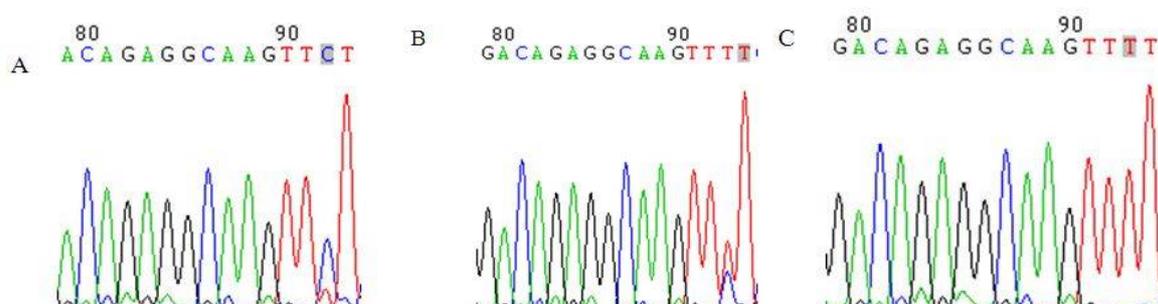


Figure 1: Schematic diagram and sequencing data of OPN. **Note:** A = C/C homozygotes; B = C/T Heterozygotes; C = T/T homozygotes

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

Variable	AS (n = 120)	Control (n = 106)	P-value
Allele, n(%)			
C	195(81.25)	189(89.15)	
T	45(18.75)	23(10.85)	0.019 ¹
Genotype, n(%)			
CC	85(70.83)	89(83.96)	
CT	25(20.83)	11(10.38)	0.019 ²
TT	10(8.34)	6(5.66)	

¹Compared with control, T vs C, $p < 0.05$; ²Compared with the controls, CT+TT vs CC, $p < 0.05$

DISCUSSION

The pathogenesis of AS remains poorly understood. However, genetic factors play a significant role [23]. Single nucleotide polymorphisms (SNP) of the human *OPN* gene have been reported to be associated with many diseases [24,25]. Based on current literature, this is the first time to report the relationship between *OPN* polymorphism and AS patients.

In this study, we have investigated the difference in *OPN* gene polymorphisms between AS patients and healthy controls. The T frequency was significantly increased in AS patients compared to the control ($p < 0.05$), the rate of the CT+TT genotype in AS patients was significantly higher than those with CC genotype compared with the control ($p < 0.05$), indicating that subjects who carried the T allele had a significantly higher risk of developing AS than those with the C allele. Dong *et al* found that the genetic variation at locus -443 of the *OPN* promoter plays important roles in the regulation of *OPN* expression and cancer progression of hepatocellular carcinoma (HCC), which is a novel determinant and target for HCC metastasis and prognosis [26]. Hao *et al* found that *OPN* -443C > T gene polymorphism may be used as a molecular marker to predict the treatment response to chemotherapy in advanced non-small-cell lung cancer patients [27]. Zhao *et al* found that *OPN* -443 C/T polymorphism is a potential predictive marker of metastasis and poor prognosis in intrahepatic cholangiocarcinoma patients [28].

So far, genetic variants in *OPN* gene have been shown to be involved in susceptibility to other immune-mediated diseases such as SLE [29,30], oligoarticular juvenile idiopathic arthritis [31] and sarcoidosis [32]. 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 [33,34] and rheumatoid arthritis [35]. However, it is first time to report the relationship between *OPN* polymorphism and AS

patients. 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 -443 polymorphism with AS.

Limitation of the study

In this study, the sample size of participants was small, thus rendering the results liable to bias. Therefore, a larger-sample study needs to be undertaken.

CONCLUSION

SNP at nt -443 of the *OPN* gene can serve as a candidate genetic marker to evaluate the risk of AS. Subjects who carry T allele may have a significantly higher risk of developing AS. This relationship is reported here for the first time.

ACKNOWLEDGEMENT

The authors thank Wei-ye Wang of Huazhong University of Science and Technology, for expert technical assistance.

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