Original Article
Association of *PTPN22* polymorphisms and ankylosing spondylitis susceptibility

Qingxi Meng¹, Xiaojun Zhang¹, Xin Liu¹, Weiguo Wang¹, Peng Yu², Qunqun Shan¹, Zhaohu Mao¹, Tingbao Zhao¹

¹Department of Spinal Cord Injury, Institute of Orthopedics and Traumatology of Chinese PLA, General Hospital of Jinan Military Area Command, Jinan, Shandong, China; ²The First Veteran Institute of Jinan Military Region, Jinan, Shandong, China

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Abstract: Background: As a susceptibility gene for AS, the polymorphisms of *PTPN22* associated with disease susceptibility. Methods: We selected two SNPs of rs1217406 and rs1217414 within *PTPN22* with Haploview software and investigated the relationship between the SNPs of *PTPN22* gene and AS susceptibility. 120 AS patients and 100 healthy people were enrolled from Qilu Hospital of Shandong University. And we genotyped the SNPs of *PTPN22* with PCR-RFLP method. Results: The results showed that C allele (rs1217406) and T allele (rs1217414) both were risk factors for AS (OR: 3.12, 2.13). The persons with A-T, C-C or C-T haplotypes were more likely to suffer AS (OR: 3.17, 3.66, 4.011). Conclusions: Due to the close relationship of *PTPN22* and AS, the study may be helpful for the early diagnosis and differential diagnosis.

Keywords: *PTPN22*, polymorphism, AS, susceptibility

Introduction

Ankylosing spondylitis (AS) is an autoimmune inflammatory disease with the clinical features of back pain and stiffness. The etiology of AS is still unknown. However, it is generally believed that genetic factor play an important role in the pathogenesis of AS. And the HLA region genes were reported to significantly associated with AS, of which human leukocyte antigen-B27 (HLA-B27) was the representative gene [1-4]. Moreover, previous studies showed that the expression level of interleukin 10 (IL10) was higher in the AS patients [5]. In addition, genes play crucial roles in regulating the expression of cytokines and immunomodulatory factors [6, 7]. Hence, we concluded that gene polymorphisms may influence the cytokine secretion pattern in patients with AS.

Protein tyrosine phosphatase nonreceptor 22 (*PTPN22*) gene encodes lymphoid protein tyrosine phosphatase (LYP) and plays a negatively regulatory role in T cell signal path. Some scholars believed that the improved activity of *PTPN22* could suppress TCR signal transduction of regulatory T cells, thus weakening the regulation function of T cells, so *PTPN22* dysfunction can lead to autoimmune diseases. Besides HLA genes, *PTPN22* is the most important susceptibility genes of human autoimmune disease and is an important candidate gene for AS in terms of autoimmune.

At present, several single nucleotide polymorphisms (SNPs) locus have been reported in *PTPN22* gene without inconsistent results. In our study, we performed gene detection of all participants including 120 AS patients and 100 healthy persons to explore whether there was relationship between polymorphisms (rs1217406, rs1217414) in promoter of *PTPN22* and AS susceptibility in Han Chinese of Shandong district.

Materials and methods

Subjects

The enrolled AS patients were from Qilu Hospital of Shandong University between 2008 to 2013 and all patients met the diagnostic criteria...
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Table 1. The characteristic of the cases and controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27.49 ± 0.84</td>
<td>26.70 ± 0.83</td>
<td>0.504</td>
</tr>
<tr>
<td>Sex</td>
<td>0.555</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>41 (34.17)</td>
<td>38 (38.00)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>79 (65.83)</td>
<td>62 (62.00)</td>
<td></td>
</tr>
</tbody>
</table>

revised in 1984 by America Rheumatism Association [8]. The participants were all Han Chinese with no blood relationship. The cases and controls were all signed the informed consent paper. All the subjects were checked and the person with other autoimmune diseases, endocrine diseases and tumor, chronic hepatitis and tuberculosis were excluded from the study.

Methods

Five ml venous blood were collected from the limosis patients and DNA was extracted with the method of EDTA anticoagulant, proteinase K digestion and salting out. The samples were stored under -20°C. 20μl PCR amplification reaction were prepared using 2× Taq PCR Masterix 10 µL, DNA template 1 µL, primer 1 µL (10 µmol/L) and DNase-free water. The PCR amplification was performed under the following conditions: 3 min 94°C initial denaturation followed by 30 cycles at 94°C for 30 s, 56°C for 30 s, 72°C for 30 s, then 72°C for 5 min. PCR products were identified with 150 g/L agarose gel electrophoresis. The primers of rs1217406 were: 5'-AAAGCCTTCACATTTTGGCATTATC-3' (forward), 5'-AGGCTTTTTCAGCGTCTTCCAACTC-3' (reverse). The primers of rs1217414 were: 5'-TATTGAGCACTTAGTATGTA-3' (forward), 5'-GCTTAGAACTGAGCTTGGTA-3' (reverse).

Enzyme digestion reaction was 20 µL with PCR products 9 µL, Rsal (10 U/µL) 0.5 µL and were put in 37°C water bath overnight. Enzyme digestion products was detected by 250 g/L agarose gel.

Statistics

Hardy-Weinberg equilibrium (HWE) was used to evaluate the representativeness of the controls. And it was completed with χ² test. T-test was used to evaluate the average age differences between cases and controls. The difference of sex distribution was calculated by χ² test. Gene counting method was used in calculating gene and allele frequencies. Odds ratio (OR) and 95% confidence intervals (95% CI) were adopted to estimate the function of SNPs on AS susceptibility with logistic regression method. The tests were all two-tailed, and P-values < 0.05 were considered statistically significant. All the test was performed in SPSS 18.0.

Results

Subject characteristics

The average age of the cases was 27.49, while the control was 26.70. There was no significant differences in age between cases and controls (P = 0.504). In the study, male participants were more than female. Male accounted for 65.83% in the cases and 62.0% in the controls. However, there was no remark differences in sex distribution among the patients and healthy people (P = 0.555) (Table 1).

Correlation analysis of PTPN22 gene polymorphisms and AS susceptibility

With genotype frequencies of rs1217406 and rs1217414 (Table 2), we found that the genotype distribution in the control was in accordance with HWE. Hence, we concluded that the selected population was representative. And we also found that there were remark differences in genotype frequencies of rs1217406 and rs1217414 between cases and controls. OR and 95% CI were calculated to estimate the association between the genotypes and AS. The results suggested that CA (OR = 3.09, 95% CI = 1.67-5.74), CC (OR = 6.64, 95% CI = 2.92-15.10) genotypes of rs1217406 and TT (OR = 5.79, 95% CI = 1.26-26.66) genotype of rs1217414 all could increase the risk for AS. In addition, the C (rs1217406) and T (rs1217414) allele carriers were more likely to suffer AS (OR = 3.12, 95% CI = 2.08-4.69 OR = 2.13, 95% CI = 1.23-2.70).

Association analysis of haplotypes and AS susceptibility

Since the linkage disequilibrium between rs1217406 and rs1217414 (D' = 0.592, r² = 0.28), we investigated the functions of four haplotypes on the AS (Table 3). The A-C haplotype accounted for a large proportion in the cases (37.08%) and controls (68.00%). The fre-
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Table 2. Association of genotypes and alleles and AS susceptibility

<table>
<thead>
<tr>
<th>Genotype/allele</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>(\chi^2)</th>
<th>(P)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1217406</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>32 (26.67)</td>
<td>59 (59.00)</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>CA</td>
<td>52 (43.33)</td>
<td>31 (31.00)</td>
<td>13.133</td>
<td>0.000</td>
<td>3.09 (1.67-5.74)</td>
</tr>
<tr>
<td>CC</td>
<td>36 (30.00)</td>
<td>10 (10.00)</td>
<td>22.701</td>
<td>0.000</td>
<td>6.64 (2.92-15.10)</td>
</tr>
<tr>
<td>A</td>
<td>116 (48.33)</td>
<td>149 (74.50)</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>124 (51.67)</td>
<td>51 (25.50)</td>
<td>31.182</td>
<td>0.000</td>
<td>3.12 (2.08-4.69)</td>
</tr>
<tr>
<td>rs1217414</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>84 (70.00)</td>
<td>81 (81.00)</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>CT</td>
<td>24 (20.00)</td>
<td>17 (17.00)</td>
<td>0.766</td>
<td>0.381</td>
<td>1.36 (0.68-2.72)</td>
</tr>
<tr>
<td>TT</td>
<td>12 (10.00)</td>
<td>2 (2.00)</td>
<td>6.286</td>
<td>0.012</td>
<td>5.79 (1.26-26.66)</td>
</tr>
<tr>
<td>C</td>
<td>192 (80.00)</td>
<td>179 (89.50)</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>T</td>
<td>48 (20.00)</td>
<td>21 (10.50)</td>
<td>7.446</td>
<td>0.006</td>
<td>2.13 (1.23-2.70)</td>
</tr>
</tbody>
</table>

Table 3. Association of haplotypes and AS susceptibility

<table>
<thead>
<tr>
<th>Haplotype site 1-site 2</th>
<th>Case 2n = 240 (%)</th>
<th>Control 2n = 200 (%)</th>
<th>(\chi^2)</th>
<th>(P) value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-C</td>
<td>89 (37.08)</td>
<td>136 (68.00)</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>A-T</td>
<td>27 (11.25)</td>
<td>13 (5.42)</td>
<td>10.775</td>
<td>0.002</td>
<td>3.17 (1.56-6.48)</td>
</tr>
<tr>
<td>C-C</td>
<td>103 (42.92)</td>
<td>43 (21.50)</td>
<td>34.062</td>
<td>0.000</td>
<td>3.66 (2.35-5.71)</td>
</tr>
<tr>
<td>C-T</td>
<td>21 (8.75)</td>
<td>8 (4.00)</td>
<td>11.297</td>
<td>0.001</td>
<td>4.01 (1.70-9.45)</td>
</tr>
</tbody>
</table>

Discussion

AS is a complex and polygenic disease based on genetic factors with more than 90% heritability [9]. Since the research on association of polymorphism of TNF-α and AS by Braun in 1995, researchers were increasingly concerned about the relationship of AS and genes [10]. Some scholars have begun to study the relationship of SNPs within PTPN22 and AS [11-14].

Single nucleotide polymorphisms (SNP) is one special form of polymorphisms, occurred in genome sequences by a single base deletion or insertion, but more often is single base substitution. SNP is usually a mutant with the from of multiple alleles or two alleles [15]. The close relationship of polymorphisms of PTPN22 and inflammation diseases were confirmed and it suggested that SNPs of PTPN22 may be causative factors for inflammation diseases including AS [16-28]. Due to the different regions and populations in the study, gene distributions were not exactly the same, thus resulting in the frequency deviation on the statistics. Our study specially selected Han population of Shandong area in China to study the correlation of PTPN22 SNPs and AS susceptibility.

With the analysis of rs1217406 and rs1217414 in 100 healthy controls, we found genotype distributions of each loci were in accordance with HWE \((P = 0.06; P = 0.34)\). And the genotype frequencies of rs1217406 and rs1217414 were significantly different between cases and controls. The CA and CC genotypes of rs1217406 both were risk factors for AS \((OR: 3.09, OR = 6.64)\). In addition, the TT genotype of rs1217414 could increase the risk for AS \((OR: 5.79)\). Further studies showed that the person with the C allele (rs1217406) or T allele (rs1217414) were more likely to suffer AS \((OR: 3.17, 2.13)\). Four haplotypes of rs1217406 and rs1217414 were analyzed in the study and the results showed three haplotypes (A-T, C-C and C-T) were closely related to AS \((OR: 3.17, 3.66, 4.011)\).

The above data could fully explain the relationship of the SNPs of PTPN22 gene and AS, so we
concluded that there existed a close relationship between polymorphisms of PTPN22 gene and AS susceptibility.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Tingbao Zhao, Department of Spinal Cord Injury, Institute of Orthopedics and Traumatology of Chinese PLA, General Hospital of Jinan Military Area Command, 25 Shifan Road, Jinan 250031, Shandong, China. Tel: 86-15263498675; E-mail: zhanghuajng@126.com

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