Original Article

IL1R1 polymorphisms are associated with ankylosing spondylitis in the Han Chinese population: a case-control study

Yuyan Na1∗, Rui Bai2∗, Yizhong Ren1, Zhenqun Zhao2, Lingyue Kong1, Ruifeng Li3, Changxu Han1, Haisheng Jia4

Departments of 1Arthroscopy and Sports Medicine, 2Pediatric Orthopedics, 3Cervical Vertebra, 4Traumatic Orthopedics, The Second Affiliated Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia Autonomous Region, China. ∗Equal contributors.

Received April 21, 2018; Accepted May 23, 2018; Epub July 1, 2018; Published July 15, 2018

Abstract: Several studies have demonstrated that polymorphisms within the IL-1 gene cluster are associated with the risk of ankylosing spondylitis (AS) in different populations. In this study, we desired to know whether IL1R1, a gene located in the IL-1 gene cluster, is a susceptible gene for AS in a Northwest Chinese Han population. The Sequenom MassARRAY assay technique was used to determine the genotype of 267 AS patients and 297 controls from Northwest China. Genotype and allele distributions of the investigated IL1R1 variants (rs10490571, rs12712127, rs956730, rs3917225, and rs3917318) were compared among the cases and controls using Chi-square/Fisher’s exact tests. In addition, the associations of these polymorphisms with AS risk were also assessed under dominant, recessive, and additive genetic models using PLINK software. We found the minor G allele of rs3917225 was associated with an increased risk of AS (OR=1.39, 95% CI: 1.09-1.77, \(P\)=0.007). Significant association was also detected for rs956730 under the dominant model (OR=0.54, 95% CI: 0.30-0.96, \(P\)=0.032) and the additive model (OR=0.55, 95% CI: 0.34-0.90, \(P\)=0.016), adjusting for age and gender. This study is the first to demonstrate the significant association between IL1R1 polymorphisms and AS susceptibility in a Northwest Chinese Han population.

Keywords: AS, IL1R1, genetic susceptibility, SNPs, case-control study

Introduction

Ankylosing spondylitis (AS) is a chronic autoimmune disease, which mainly affects the sacroiliac joints and spine, causing bone and joint erosion and even ankylosis. AS is highly heritable with an approximate prevalence of three out of every 1000 adults in the Chinese population, and it often occurs in young men aged 20-30 with a higher family aggregation [1, 2]. The precise pathogenesis of AS has not been illustrated, but some investigations have suggested that hereditary factors are related to the predisposition of AS in the Han Chinese population. Common variants in ETS1, ERAP1, IL12B, PTGER4, JARID1A, and JMY may contribute to AS susceptibility [3-6].

It has been shown that Interleukin 1β (IL-1β), the active form of IL-1 in inflammation, as a pleiotropic cytokine might be involved in the active inflammation of AS. Chou et al. suggested that patients had increased production of IL-1β from peripheral blood mononuclear cells during active inflammation of AS [7]. Vazquez and colleagues showed that levels of IL-1β were higher in patients with AS than in healthy controls [8]. Single nucleotide polymorphism (SNP) markers within the IL1 gene complex members (IL1A, IL1B, IL1RN) were found to be significantly correlated with AS in Taiwanese Chinese and in three Canadian populations [9, 10].

Although IL1 was thought to be correlated with AS, whether its receptor (IL1R1) was also implicated in the pathogenesis of AS remained unknown. Thus, the association of IL1R1 polymorphisms with AS risk provides a new direction for further research. In this study, we randomly selected five IL1R1 polymorphisms
IL1R1 polymorphisms and AS susceptibility

Materials and methods

Subjects

This study was carried out in accordance with the Helsinki Declaration, and the research design was approved by the Ethics Committee of the Second Affiliated Hospital of Inner Mongolia Medical University. A total of 267 AS patients and 297 ethnically and geographically matched healthy controls were enrolled in the two hospitals from March 2013 to March 2016. The AS patients were diagnosed according to the modification of the 1984 New York criteria [11]. The controls were selected from a physical examination center and included individuals without a personal or family history of AS disorder. All the patients and controls provided their written informed consent after a full explanation for the genetic study.

DNA extraction and SNPs genotyping

Venous blood collection was conducted from all participants in the two hospitals. Using the GoldMag-Mini Purification Kit (GoldMag Co. Ltd. Xi’an, China), genomic DNA was extracted from leukocytes of the blood samples following a standard protocol. Then the DNA concentration was measured by the NanoDrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA) at a wavelength value of A260 and A280 nm. Next, DNA samples were stored at -20°C before genotyping. We selected five IL1R1 variants (rs10490571, rs12712127, rs956730, rs3917225, and rs3917318) with minor allele frequencies > 5% in the Chinese Han Beijing population in the HapMap database (http://www.hapmap.org) for genotyping. The five SNPs were chosen randomly and included those not reported to be associated with OA susceptibility.

Statistical analysis

All statistical analyses were performed using Microsoft Excel and SPSS 16.0 (SPSS, Chicago IL USA). Differences in age and gender among the AS patients and healthy controls were evaluated by Welch’s t test and Pearson’s χ² test, respectively. Deviation from the Hardy-Weinberg equilibrium (HWE) of allele frequency of IL1R1 rs10490571, rs12712127, rs956730, rs3917225, and rs3917318 in controls was tested by the exact test. Genotype and allele distributions of each SNP were compared among the cases and controls using unconditional logistic regression analysis with or without adjustment for age and gender and Chi-square/Fisher’s exact tests [14], respectively. The associations of these SNPs with AS risk were also assessed under dominant, recessive, and additive genetic models using
PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) [15]. An odds ratio (OR) and 95% confidence intervals (CI) were used to evaluate the effect of each polymorphism and AS risk [16]. Statistical significance was set at a two-sided \( P < 0.05 \).

### Results

A total of 267 AS patients and 297 controls were genotyped for \( IL1R1 \) variants in the present study (Table 2). There were statistically significant differences between AS patients and controls in terms of age (\( P < 0.001 \)) and gender (\( P < 0.001 \)). So, unconditional logistic regression analysis with or without adjustment for age and gender was adopted to calculate the odds ratios. Besides \( IL1R1 \) rs12712127, the other four SNPs were all in line with HWE in the controls (\( P > 0.05 \)).

The minor allele frequencies of the \( IL1R1 \) polymorphisms in cases and controls are listed in Table 3. A significant difference was observed in the rs3917225 allele distribution between the AS patients and the healthy controls (41.4% versus 33.7%). And this locus was significantly associated with an increased risk of AS (OR=1.39, 95% CI: 1.09-1.77, \( P=0.007^* \)). Further-
more, significant association with increased AS susceptibility was also found in the “GG” genotype of rs3917225 when it was compared to the wild “AA” genotype (OR=1.95, 95% CI: 1.16-3.26, \( P=0.029 \)). However, multivariate unconditional logistic regression analysis with adjustment by age and gender did not reveal any sig-

### Table 3. Basic informations on candidate \( IL1R1 \) polymorphisms

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Chromosome</th>
<th>Position</th>
<th>Allele</th>
<th>Minor allele frequency</th>
<th>HWE</th>
<th>OR (95% CI)</th>
<th>( P^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10490571</td>
<td>IL1R1</td>
<td>2q12.1</td>
<td>102717337</td>
<td>T/C</td>
<td>0.193</td>
<td>0.409</td>
<td>1.18 (0.87-1.60)</td>
<td>0.284</td>
</tr>
<tr>
<td>rs12712127</td>
<td>IL1R1</td>
<td>2q12.1</td>
<td>102726661</td>
<td>G/A</td>
<td>0.228</td>
<td>0.000</td>
<td>1.12 (0.84-1.49)</td>
<td>0.433</td>
</tr>
<tr>
<td>rs956730</td>
<td>IL1R1</td>
<td>2q12.1</td>
<td>102758116</td>
<td>A/G</td>
<td>0.232</td>
<td>0.769</td>
<td>0.83 (0.63-1.08)</td>
<td>0.170</td>
</tr>
<tr>
<td>rs3917225</td>
<td>IL1R1</td>
<td>2q12.1</td>
<td>102769302</td>
<td>G/A</td>
<td>0.414</td>
<td>1.000</td>
<td>1.39 (1.09-1.77)</td>
<td>0.007*</td>
</tr>
<tr>
<td>rs3917318</td>
<td>IL1R1</td>
<td>2q12.1</td>
<td>102792760</td>
<td>G/A</td>
<td>0.455</td>
<td>0.064</td>
<td>0.89 (0.71-1.13)</td>
<td>0.341</td>
</tr>
</tbody>
</table>

HWE: Hardy-Weinberg equilibrium; OR: odds ratio; 95% CI: 95% confidence interval. \( P^a \) \( P \leq 0.05 \) indicates statistical significance.

### Table 4. Associations between \( IL1R1 \) rs3917225 and rs956730 and AS susceptibility under multiple inheritance models

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Models</th>
<th>Genotype</th>
<th>Genotype</th>
<th>Cases</th>
<th>Controls</th>
<th>Without adjustment</th>
<th>With adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3917225 (A &gt; G)</td>
<td>Genotype</td>
<td>AA</td>
<td>93</td>
<td>131</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA</td>
<td>127</td>
<td>132</td>
<td>1.36 (0.95-1.94)</td>
<td>0.029*</td>
<td>1.33 (0.72-2.44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>47</td>
<td>34</td>
<td>1.95 (1.16-3.26)</td>
<td>0.029*</td>
<td>1.69 (0.72-4.00)</td>
</tr>
<tr>
<td></td>
<td>Dominant</td>
<td>AA</td>
<td>93</td>
<td>131</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG+GA</td>
<td>174</td>
<td>166</td>
<td>1.48 (1.05-2.08)</td>
<td>0.029*</td>
<td>1.41 (0.79-2.50)</td>
</tr>
<tr>
<td></td>
<td>Recessive</td>
<td>AA+GA</td>
<td>220</td>
<td>263</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>47</td>
<td>34</td>
<td>1.65 (1.03-2.66)</td>
<td>0.037*</td>
<td>1.46 (0.66-3.23)</td>
</tr>
<tr>
<td></td>
<td>Additive</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.39 (1.09-1.76)</td>
<td>0.008*</td>
<td>1.31 (0.87-1.96)</td>
</tr>
<tr>
<td>rs956730 (G &gt; A)</td>
<td>Genotype</td>
<td>GG</td>
<td>156</td>
<td>158</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AG</td>
<td>98</td>
<td>119</td>
<td>0.83 (0.59-1.18)</td>
<td>0.380</td>
<td>0.59 (0.32-1.06)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>13</td>
<td>20</td>
<td>0.66 (0.32-1.37)</td>
<td>0.26</td>
<td>0.26 (0.07-1.04)</td>
</tr>
<tr>
<td></td>
<td>Dominant</td>
<td>GG</td>
<td>156</td>
<td>158</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA+AG</td>
<td>111</td>
<td>139</td>
<td>0.81 (0.58-1.13)</td>
<td>0.210</td>
<td>0.54 (0.30-0.96)</td>
</tr>
<tr>
<td></td>
<td>Recessive</td>
<td>GG+AG</td>
<td>254</td>
<td>277</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>13</td>
<td>20</td>
<td>0.71 (0.35-1.45)</td>
<td>0.340</td>
<td>0.33 (0.09-1.28)</td>
</tr>
<tr>
<td></td>
<td>Additive</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.82 (0.63-1.08)</td>
<td>0.160</td>
<td>0.55 (0.34-0.90)</td>
</tr>
</tbody>
</table>

\( P \) values were calculated by unconditional logistic regression analysis with or without adjustments for age and gender. \( * P \leq 0.05 \) indicates statistical significance.
significant correlation between the genotype of these SNPs and a risk for OA (Table 4).

Furthermore, the potential associations between these SNPs and AS susceptibility were also investigated under dominant, recessive, and additive genetic models. Significant evidence was detected for rs956730 under the dominant model (OR=0.54, 95% CI: 0.30-0.96, P=0.032) and the additive model (OR=0.55, 95% CI: 0.34-0.90, P=0.016) with adjustment by age and gender (Table 4). However, there was no significant difference after the Bonferroni correction.

Discussion

In this investigation, a case-control study was designed to investigate the association of IL1R1 polymorphisms with AS risk in a Northwest Chinese Han population. Our findings showed that the gene of IL1R1 was correlated with AS, and that rs3917225 and rs956730 might be risk associated SNPs that are involved in the pathogenesis of this disease, but the detailed mechanism is still not well established.

IL-1β has proinflammatory action, which is mediated by some transcriptional factors, such as Mitogen-activated protein kinase and nuclear factor kB [17, 18]. Studies also found that IL-1β could trigger the production of matrix metalloproteases with subsequent subchondral erosion and suppression of chondrocyte proteoglycan synthesis [19, 20]. Analyses of peripheral blood mononuclear cells by an ELISA test indicated an increase in IL-1β levels in AS patients compared with healthy controls [8]. Therefore, we speculated IL-1β might influence the pathogenesis of AS by activating inflammation and the subsequent cartilage lesion.

IL1R1, located in the IL-1 gene cluster on chromosome 2q, which encodes cytokine receptor IL1R1, which can combine with IL-1 on the cell surface and affect NF-κB signaling, leading to the up-regulation of inflammatory and immune gene expression [21]. And the degree of IL1R1 expression on the cell surface affects the response of cells to IL-1 [22]. A genetic basis, such as IL1R1 polymorphisms, may bring about interindividual differences in IL1R1 receptor production. In the present study, we found IL1R1 rs956730 was a protective factor for AS and rs3917225 was an increased risk factor for AS in a Northwest Chinese Han population, which may be because these SNPs affect the expression of the IL1R1 gene and eventually influence the activity of the inflammatory and immune reactions.

This study, to our knowledge, is the first to present IL1R1 polymorphisms and AS susceptibility in a Northwest Chinese Han population. Walter et al. indicated that SNPs within the IL-1 gene cluster (IL1A, IL1B, IL1RN) are associated with susceptibility to AS in three Canadian populations. And one year later, Chou and colleagues also found significant correlations between the IL-1 gene cluster polymorphisms and AS risk in Taiwanese Chinese. However, neither of the two studies has investigated the potential association between the SNPs of IL1R1, a gene also located in the IL-1 gene cluster, and AS risk. In the present study, we found IL1R1 polymorphisms are risk factors for AS in a Northwest Chinese Han population, which is in accordance with the findings of these two studies.

Several potential limitations must be considered when interpreting the results of this study. First, the sample size (267 AS patients and 297 controls) in this investigation is small, which may influence the stability of our results. Second, there may be other SNPs in this gene that are related to AS susceptibility but that were not evaluated for their potential associations.

To sum up, this study is the first to demonstrate the significant association between IL1R1 polymorphisms and AS susceptibility in a Northwest Chinese Han population. Given that AS is a prevalent disease in young men worldwide, identifying potential predictive markers for this disease is of great significance for diagnosing and treating AS in the general population. Therefore, future studies should focus on the validation of this association in other populations, using a larger sample size.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81460331; 81560349; 81660457).

Disclosure of conflict of interest

None.
IL1R1 polymorphisms and AS susceptibility

Address correspondence to: Haisheng Jia, Department of Traumatic Orthopedics, The Second Affiliated Hospital of Inner Mongolia Medical University, Hohhot 010030, Inner Mongolia Autonomous Region, China. E-mail: 15591886880@163.com

References


15. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ and Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81: 559-575.


IL1R1 polymorphisms and AS susceptibility
