CD226 rs763361 (Gly307Ser) Polymorphism Is Associated with Susceptibility to Rheumatoid Arthritis in Zahedan, Southeast Iran

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease with many genetic factors predisposing to disease susceptibility. The aim of the present study was to investigate the impact of CD226 rs727088 and rs763361 polymorphisms and susceptibility to RA in a sample of the Iranian population. Methods: This case-control study was carried out on 100 patients with RA and 104 healthy subjects. The polymorphisms were determined using tetra amplification refractory mutation system-polymerase chain reaction assay. Results: The rs763361 (Gly307Ser) polymorphism increased the risk of RA in codominant, dominant and recessive-tested inheritance models (odds ratio [OR] = 3.18, 95% confidence intervals [95% CI] = 1.44-7.02, \(P = 0.004\), CC vs. TT, and OR = 1.98, 95% CI = 1.10-3.57, \(P = 0.023\), CC vs. CT-TT, and OR = 2.61, 95% CI = 1.26-5.37, \(P = 0.010\), CC + CT vs. TT, respectively). In addition, the rs763361 T allele increased the risk of RA (OR = 2.06, 95% CI = 1.38-3.08, \(P <0.001\)). However, no significant difference was observed among the groups regarding CD226 rs727088 polymorphism (\(\chi^2 = 3.20, P = 0.202\)). Conclusions: Our finding showed that CD226 rs763361, but not rs727088, gene polymorphism increased the risk of RA in a sample of the Iranian population. Iran. Biomed. J. 17 (4): 194-199, 2013

Keywords: Rheumatoid arthritis (RA), CD226, Polymorphism

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease of unknown etiology. RA is characterized by inflammation and cell proliferation in the synovial lining of joints that eventually leads to cartilage and bone destruction. Both genetic and environmental factors have been shown to be relevant, contributory factors to the expression and complications of this disease [1, 2]. The prevalence of RA is about 1% of the population worldwide, and genetic factors have been estimated to account for 60% of the disease risk [3].

D226 gene, which is located on chromosome 18q22.3, is composed of 7 exons. The CD226 (DNAM-1) is a 67-kDa type I transmembrane glycoprotein and a member of the immunoglobulin superfamily [4]. CD226 mediates cell activation and differentiation and is expressed on the majority of immune cells, including natural killer cells, T-cells, monocytes, and platelets [4, 5]. There are some evidences regarding the role of CD226 rs763361 polymorphism in autoimmune diseases such as type I diabetes, multiple sclerosis, autoimmune thyroid disease, Wegener’s granulomatosis, psoriasis, and RA [6-11]. It has been proposed that the rs727088 polymorphism in 3’-UTR of CD226 has a functional influence on CD226 transcription levels [12]. CD226 rs763361 (Gly307Ser) non-synonymous polymorphism could interfere in the phosphorylation of CD226 at 322Tyr and 329Ser residues, and the downstream signal transduction may be modified by these

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posttranslational modifications [13, 14]. Genetic risks may differ among different populations [15]. Therefore, repeating previously reports of association of CD226 polymorphisms and RA in other population is desired to find out the genetic risk in our population.

The present study was aimed to evaluate the impact of CD226 rs763361 (Gly307Ser) and rs727088 polymorphisms on the susceptibility to RA in a sample of the Iranian population.

**MATERIALS AND METHODS**

**Patients.** We investigated the possible association between rs727088 and rs763361 polymorphisms of CD226 and RA susceptibility in 100 patients (87 female and 13 male with an average age of 44.7 ± 13.4 years), fulfilling the American College of Rheumatology criteria for RA [16]. All the subjects were patients of the Rheumatology Clinic at Zahedan University of Medical Sciences [2, 15, 17]. The control group consisted of 104 healthy individual (67 female and 37 male) with a mean age of 44.7 ± 9.7 years and unrelated to RA patients. The Ethics Committee of Zahedan University of Medical Sciences (Zahedan, Iran) approved the project, and an informed consent was obtained from all patients and healthy individuals. Blood samples from patients and healthy control were collected in Na-EDTA tubes. Genomic DNA of each individual was extracted from peripheral blood samples as described previously [15].

The CD226 genomic sequences (NT_025028) were obtained from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). We searched the polymorphisms and designed the primers for tetra amplification refractory mutation system-polymerase chain reaction assay according to Ye et al. [18] procedure. This method is a simple and rapid method for detection of single nucleotide polymorphism [18-20] (Table 1).

| Table 1. Primers sequence for detection polymorphisms of CD226 rs763361 and rs727088 |
|--------------------------------------------------|-----------------|-----------------|-----------------|
| Primers  | Sequence (5’ to 3’ Amplicon | size (bp) |
| rs763361 C>T | FO TTGCATAAAAGATCCATGCATGAGTAC 385 |
| | RO GATTTCCTGTGCATCTCAGTCAAGAA |
| | FI (T allele) CAT GGATTGATTGGTAGGTTGCCT 251 |
| | RI (C allele) CCAATACTATAGAAGTCCCATCTCTACG 187 |
| rs727088 G>A | FO TGTCATTAGGGCTGTCTTTGTCTGAATAG 342 |
| | RO CCAGGTCTAGCCTTAGGAGCAAATGTA |
| | FI (G allele) TTCCCTCCAAAAATTITACCTACCAACG 235 |
| | RI (A allele) AGTGACAGTTGAAAGTGGTGGCATATAT 161 |

FO, forward outer; RO, reverse outer; FI, forward inner; RI, reverse inner.

**RESULTS**

There was no significant difference among groups regarding age (\(P = 0.815\)), but the sex was significantly different (\(P<0.05\)). Table 2 shows the genotype and allele frequencies of the non-synonymous polymorphism rs763361 of the CD226 gene in RA patients and in controls. Significant differences were observed in genotype frequencies among the groups.
regarding CD226 rs763361 polymorphisms ($\chi^2 = 10.25, P = 0.006$).

The rs763361 variant increased the risk of RA in codominant, dominant and recessive-tested inheritance models (OR = 3.18, 95% CI = 1.44-7.02, $P = 0.004$, CC vs. TT, OR = 1.98, 95% CI = 1.10-3.57, $P = 0.023$, CC vs. CT-TT, and OR = 2.61, 95% CI = 1.26-5.37, $P = 0.010$, CC + CT vs. TT, respectively) (Table 2). Moreover, the distribution frequency of the rs763361 T allele was significantly higher in RA in comparison with the control group (49.5% vs. 32.2%, respectively), and the T allele increased the risk of RA (OR = 2.06, 95% CI = 1.38-3.08, $P<0.001$).

The genotype in CD226 rs763361 in control group was in HWE ($\chi^2 = 3.57, P = 0.059$), while in RA was out of HWE ($\chi^2 = 6.75, P = 0.009$). No significant differences were found in genotype or allelic frequencies between cases and controls regarding rs727088 polymorphism of CD226 ($\chi^2 = 3.20, P = 0.202$). The rs727088 polymorphism was not associated with RA in any tested inheritance models (Table 3). CD226 rs727088 genotypes in normal and cases were in HWE ($\chi^2 = 0.001, P = 0.973$ and $\chi^2 = 0.53, P = 0.465$, respectively).

**DISCUSSION**

In the present study, the association of CD226 rs763361 and rs727088 gene polymorphisms with RA in a sample of the Iranian population has been evaluated. We showed that the non-synonymous (Gly307Ser) variant, rs763361 polymorphism, is associated with RA in our population. No significant association was found between rs727088 polymorphism and RA.

**Table 2.** Genotype and allele frequency distribution of CD226 rs763361 polymorphism in rheumatoid arthritis (RA) patients and healthy subjects

<table>
<thead>
<tr>
<th>rs763361 C&gt;T</th>
<th>RA n (%)</th>
<th>Control n (%)</th>
<th>$^a$OR (95%CI)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>32 (32.0)</td>
<td>52 (50.0)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>CT</td>
<td>37 (37.0)</td>
<td>37 (35.6)</td>
<td>1.52 (0.79-2.92)</td>
<td>0.213</td>
</tr>
<tr>
<td>TT</td>
<td>31 (31.0)</td>
<td>15 (14.4)</td>
<td>3.18 (1.44-7.02)</td>
<td>0.004</td>
</tr>
<tr>
<td>Dominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>32 (32.0)</td>
<td>52 (50.0)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>CT + TT</td>
<td>68 (68.0)</td>
<td>47 (50.0)</td>
<td>1.98 (1.10-3.57)</td>
<td>0.023</td>
</tr>
<tr>
<td>Recessive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC + CT</td>
<td>69 (69.0)</td>
<td>89 (85.6)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>TT</td>
<td>31 (31.0)</td>
<td>15 (14.4)</td>
<td>2.61 (1.26-5.37)</td>
<td>0.010</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>101 (50.5)</td>
<td>141 (67.8)</td>
<td>reference</td>
<td>-</td>
</tr>
<tr>
<td>T</td>
<td>99 (49.5)</td>
<td>67 (32.2)</td>
<td>2.06 (1.38-3.08)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a$adjusted for sex and age; OR, odds ratio; CI, confidence intervals

http://IBJ.pasteur.ac.ir
Table 3. Genotype distribution of \textit{CD226} rs727088 polymorphism in rheumatoid arthritis (RA) patients and normal subjects

<table>
<thead>
<tr>
<th>rs727088 G&gt;A</th>
<th>RA n (%)</th>
<th>Control n (%)</th>
<th>(^{a})OR (95%CI)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>29 (29.0)</td>
<td>23 (22.1)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>GA</td>
<td>53 (53.0)</td>
<td>52 (50.0)</td>
<td>1.0 (0.50-1.99)</td>
<td>0.997</td>
</tr>
<tr>
<td>AA</td>
<td>18 (18.0)</td>
<td>29 (27.9)</td>
<td>0.73 (0.31-1.72)</td>
<td>0.467</td>
</tr>
<tr>
<td>Dominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>29 (29.0)</td>
<td>23 (22.1)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>GA+AA</td>
<td>71 (71.0)</td>
<td>81 (77.9)</td>
<td>1.09 (0.56-2.11)</td>
<td>0.802</td>
</tr>
<tr>
<td>Recessive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG+GA</td>
<td>82 (82.0)</td>
<td>75 (72.1)</td>
<td>reference</td>
<td>-</td>
</tr>
<tr>
<td>AA</td>
<td>18 (18.0)</td>
<td>29 (27.9)</td>
<td>0.74 (0.36-1.49)</td>
<td>0.391</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>111 (55.5)</td>
<td>98 (47.1)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td>89 (44.5)</td>
<td>110 (52.9)</td>
<td>0.71 (0.48-1.05)</td>
<td>0.093</td>
</tr>
</tbody>
</table>

\(^{a}\)adjusted for sex and age; OR, odds ratio; CI, confidence intervals

Our results regarding rs763361 polymorphism is in agreement with the findings of DU et al. [11], which have found that the rs763361 variant in the \textit{CD226} gene is significantly associated with RA in the Chinese population. In addition, a meta-analysis performed by Du et al. [11] showed an association between rs763361 and RA in both the Chinese and the Colombian populations. The test of OR heterogeneity indicated that rs763361 may play a more important role in non-European populations in comparison with the European population [11].

Maiti et al. [21] demonstrated that the coding variant rs763361 in \textit{CD226} gene is associated with multiple autoimmune diseases such as RA, celiac disease, and systemic lupus erythematosus in the non-European populations. Suzuki et al. [10] have found that Gly307Ser (rs763361) in \textit{CD226} is associated with susceptibility to RA in Japanese patients. Maiti et al. [21] and Hafler et al. [6] have revealed that \textit{CD226} Gly307Ser variant is associated with susceptibility to RA and multiple autoimmune diseases. In contrast to our findings, Liu et al. [22] did not find any association between \textit{CD226} rs763361 polymorphism and RA susceptibility in a Chinese population.

Antitumor necrosis factor therapy has been used for treatment of RA, although 30-40% of patients have little or no response. Tan et al. [23] have found that the \textit{CD226} rs763361 C allele conferred reduced response to treatment. The result proposed that \textit{CD226} gene polymorphisms, which increased the risk of RA, have an additional role in influencing the response to antitumor necrosis factor treatment.

\textit{CD226} rs763361 variant has been reported to be associated with type 1 diabetes, multiple sclerosis, autoimmune thyroid disease, Wegener’s granulomatosis, psoriasis, RA and primary Sjogren’s syndrome [6-11, 21]. There is little data on the contribution of \textit{CD226} rs727088 variant and disease susceptibility.

It has been reported that \textit{CD226} rs727088 variant, located in the 3’-untranslated region, is associated with impaired expression of \textit{CD226} in T and natural killer T cells and is associated with susceptibility to systemic lupus erythematosus [12]. Bossini-Castillo et al. [24] reported that the rs763361, rs34794968, and rs727088 tested genetic variants do not individually influence systemic sclerosis susceptibility but a \textit{CD226} three-variant haplotype is associated with genetic susceptibility to systemic sclerosis-related pulmonary fibrosis. No significant association was found among \textit{CD226} polymorphisms, rs727088, rs34794968, and rs763361 as well as giant cell arthritis [25]. In the present study, we did not find any association between \textit{CD226} rs727088 polymorphism and RA in a sample of Iranian individuals.

\textit{CD226} molecule is expressed on the majority of immune cells including natural killer cells and T cells mediating their activation and differentiation [4]. Interaction of \textit{CD226} with its ligands results in a variety of cellular responses including innate and adaptive immunity [26]. Furthermore, phosphorylation of the cytoplasmic domain of the \textit{CD226} molecule assists in co-localization with leukocyte function-associated antigen 1 and T-cell activation [13].

The findings of our study may be limited by relatively small sample sizes and the statistically significant differences between cases and controls regarding sex. However, this difference probably does not have a significant impact on the results, because we used sex as a covariate in regression analysis.

To the best of our knowledge, this is the first report regarding the association between \textit{CD226} poly-
morphisms and RA in a sample of the Iranian population. We found a significant association between non-synonymous variant (Gly307Ser), rs763361 polymorphism, in CD226 and susceptibility to RA. Furthermore, association studies with large sample size and different ethnicities are needed to confirm our findings.

ACKNOWLEDGMENTS

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REFERENCES


