

## 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 ( $\chi^2 = 3.20$ ,  $P = 0.072$ ). **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 glycol-

protein 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 1 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

**Table 1.** Primers sequence for detection polymorphisms of CD226 rs763361 and rs727088

Primers	Sequence (5' to 3')	Amplicon size (bp)
rs763361 C>T		
FO	TTGCATAAAGATCCATGCATGAGTAC	385
RO	GATTTCTGTTGCATCTCAGTCAAGAA	
FI (T allele)	CATGGATTGATTGGTAGGTTGCCT	251
RI (C allele)	CCAATAACTATAGAAGTCCCATCTCTAACG	187
rs727088 G>A		
FO	TGTCATTAGGGCTGTCTTTGTCTGAATAG	342
RO	CCAGGTCTAGCCTTAGGAGCAAATGTA	
FI (G allele)	TTCCCTCCCAAATTTCTACCCTAACG	235
RI (A allele)	AGTGACAGTTGAAAGTGGTGGCATAGTAT	161

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

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  $44.7 \pm 13.4$  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  $44.4 \pm 9.7$  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).

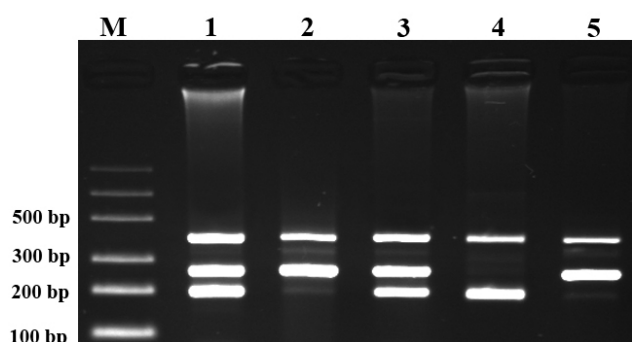
PCR was performed by using commercially available PCR premix (AccuPower PCR PreMix; BIONEER, Daejeon, Korea) according to the manufacturer's instructions. Briefly, 1  $\mu$ L template DNA (~100 ng/ $\mu$ L), 1  $\mu$ L of each primer (10 pmol/ $\mu$ L), and 15  $\mu$ L DNase-free water were added to AccuPower PCR PreMix.

Amplification was performed with an initial denaturation step at 95°C for 5 min, followed by 30 cycles of 30 s at 95°C, 30 s at 64°C for rs727088, 23 s at 60°C for rs763361 as well as 23 s at 72°C for rs727088 and 25 s at 72°C for rs763361 with a final step at 72°C for 10 min. PCR products were verified on a 2.0% agarose gel containing 0.5  $\mu$ g/ml ethidium bromide, and photographs was taken (Figures 1 and 2). To confirm genotyping quality, all polymorphisms in random samples were re-genotyped.

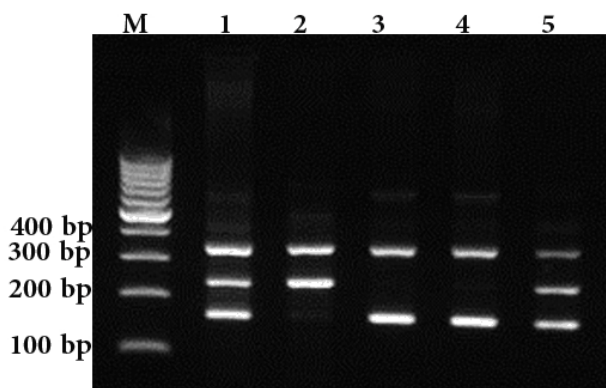
**Statistical analysis.** Statistical analysis was performed using SPSS version 18 software. We estimated the Hardy-Weinberg equilibrium (HWE) separately for cases and controls. The associations between genotypes of CD226 gene and RA were assessed by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses adjusted for sex and age.

## 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



**Fig. 1.** Photograph of the PCR products of CD226 rs763361 C>T polymorphism. In tetra amplification refractory mutation system-polymerase chain reaction method, two external primers (control band) and two inner primers (allele specific primers) were used. The product sizes were 187 bp for C allele, 251 bp for T allele, and 385 bp for control band. M, DNA Marker; lanes 1 and 3, rs763361 CT; lanes 2 and 5, TT, and lane 4, CC.



**Fig. 2.** Photograph of the PCR products of CD226 rs727088 G>A polymorphism by using tetra amplification refractory mutation system-polymerase chain reaction method. Product sizes were 161 bp for A allele, 235 bp for G allele, and 342 bp for control band. M, DNA Marker; Lanes 1 and 5, rs727088 GA; lane 2, GG, and lanes 3 and 4, AA.

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 ( $\chi^2 = 3.20$ ,  $P = 0.020$ ). 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 rs727088 and rs763361 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

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

<sup>a</sup>adjusted for sex and age; OR, odds ratio; CI, confidence intervals

**Table 3.** Genotype distribution of CD226 rs727088 polymorphism in rheumatoid arthritis (RA) patients and normal subjects

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

<sup>a</sup>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 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 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 CD226 is associated with susceptibility to RA in Japanese patients. Maiti *et al.* [21] and Hafler *et al.* [6] have revealed that 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 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 CD226 rs763361 C allele conferred reduced response to treatment. The result proposed that CD226 gene polymorphisms, which increased the risk of RA, have an additional role in influencing the response to antitumor necrosis factor treatment.

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 CD226 rs727088 variant and disease susceptibility.

It has been reported that CD226 rs727088 variant, located in the 3'-untranslated region, is associated with impaired expression of 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 CD226 three-variant haplotype is associated with genetic susceptibility to systemic sclerosis-related pulmonary fibrosis. No significant association was found among CD226 polymorphisms, rs727088, rs34794968, and rs763361 as well as giant cell arthritis [25]. In the present study, we did not find any association between CD226 rs727088 polymorphism and RA in a sample of Iranian individuals.

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 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 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 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.

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