Association of IL-10 rs1800896 (-1082 G/A) gene polymorphism and susceptibility to *rheumatoid arthritis* (RA) in Northeast of Iran

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Abstract

Background and objectives: Rheumatoid arthritis (RA) is a complex and systemic inflammatory disease in which the immune response is disturbed. *Single nucleotide polymorphisms* (SNPs) in the promoter regions of regulatory cytokines including *interleukin-10* (IL-10) may lead to exacerbated immune response and increased risk of RA. Here, we aimed to assess the association of IL-10 -1082 (G/A) (rs1800896) promoter polymorphism with the susceptibility to RA in a population in northeast of Iran.

Methods: A total of 130 RA patients and 128 sex- and age- matched healthy donors were enrolled. The polymerase chain reaction (PCR) was used to amplify the polymorphic regions and restriction fragment length polymorphism (RFLP) technique was applied to detect rs1800896. SPSS 22.0 software was used to analyze data statistically.

Results: Our findings revealed that G allele was significantly associated with the increased risk of RA [OR = 1.88, 95% CI (1.32–2.66), P-value = 0.0001] in patients. Setting AA genotype as the reference, the AG [OR = 2.93, 95% CI (1.68–5.12), P-value = 0.0001] and GG [OR = 5.73, 95% CI (2.30–14.23), P-value = 0.0001] genotypes were significantly associated with RA susceptibility.

Conclusion: The present study suggests that the IL-10 -1082 (G/A) genetic variants are associated with RA susceptibility, but not with the disease activity. While this is the first time to report such an association in a population in northeast of Iran, further studies are needed to confirm these findings.

Introduction

Rheumatoid arthritis (RA) is a complex, chronic and systemic inflammatory disease in which the immune tolerance is breached (1). RA could result in a progressive destruction of affected joints and organs by involving the synovial fluid (2). RA may affect up to 1% of the adult population and is known as an increasing health burden worldwide which could be associated with limitations in daily living activities in elderlies (3).

The exact etiology of RA is not completely understood. However, the genetic and environmental components have been proposed to be involved in determining the susceptibility of the disease (4,5), of which the genetic factors may account for approximately up to 60% of RA pathogenesis (4). The risk alleles at various genetic loci have shown a great potential of heritability which may be accounted as predisposing factors to RA development (6). The most powerful and widely studied genetic factors within immune system which have been considered as RA susceptibility factors are *human leukocyte antigen* (HLA) class II molecules and related genetic loci (7). Moreover, linkage-based approaches to assess genetic associations with RA have introduced other genes outside the HLA region including cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) (8), PTN22 (9), STAT4 (10,11), peptidyl arginine deiminase type IV (PADI4) (12,13) and pro- or anti- inflammatory cytokines.

Cytokines are of the most important signaling molecules which play a crucial role in the immune response and may be disturbed in autoimmune disorders including RA (14). *Single nucleotide polymorphisms* (SNPs) in the promoter regions of regulatory cytokine may lead to aberrant changes in the expression levels and exacerbated innate or adaptive immune response in inflammatory conditions [15-18]. Therefore, several studies have investigated the association of the genetic variants in cytokines including tumor necrosis factor-alpha (TNF-α) (15), transforming growth factor-β (TGF-β) (19), interleukin-6 (IL-6) (20), interferon-gamma (IFN-γ) (21) and interleukin-10 (IL-10) (18,22) with RA.

IL-10 is a multifunctional cytokine which is normally produced by activated macrophages and B lymphocytes. IL-10 is known to possess anti-inflammatory characteristics by inhibiting the production of pro-inflammatory cytokines (23). However, the elevated levels of IL-10 have been also reported and have been associated with the increased activity of RA in several studies (24). Therefore, the altered levels of IL-10 could be associated with the susceptibility to RA. Although several studies have addressed the association between IL-10 gene promoter polymorphisms including -1082 (G/A) SNP and RA susceptibility in different regions and ethnic groups (17,18,25,22,26,27), the results have been controversial. Moreover, there is still lack of association studies between -1082 (G/A) genetic variants and RA susceptibility in Iran. Therefore, we aimed to assess the association of -1082 (G/A) (rs1800896) genetic variant with the susceptibility to RA in an Iranian population in comparison to normal subjects.

Materials and Methods

Patients and controls

A total of 128 patients fulfilling the revised criteria of the American College of Rheumatology (ACR) for RA and 130 age- and sex-matched healthy subjects were enrolled in this study from Rheumatology Research Center, Sayyad Shirazi educational hospital, Gorgan, Iran. The diagnosis was confirmed and Disease Activity Scale (DAS) 28a was calculated by an expert rheumatology specialist. A written informed consent following the declaration of Helsinki was obtained from all individuals. A volume of 5mL whole blood was taken from all participants and transferred to the laboratory. PBMCs were separated using Ficoll-Paque (Baharafshan, Tehran, Iran) gradient centrifugation, following previously described methods (28) and stored at -80 ºC until DNA extraction.

DNA extraction and genotyping

Genomic DNA was extracted using a DNA isolation kit (Dena Zist, Iran) from the previously stored PBMCs and kept at -20 ℃ until use. The polymerase chain reaction (PCR) was used to amplify the polymorphic regions and restriction fragment length polymorphism (RFLP) technique was applied to detect IL-10 promoter SNP at the positions of -1082 (G/A). The PCR was performed in a final volume of 25 mL reaction mixture containing 50 ng of template DNA, 10X PCR buffer (Bioron, Germany), 2 mM MgCl2, 0.4 μM forward primer (5'-CTCGCCGCAACCCAACTGGC -3'), 0.4 mM reverse primers (5'-TCTTACCTATCCCTACTTCC -3') and 1.5 U *Taq* polymerase (Bioron, Germany) as follows: initial denaturation at 95 ℃ for 5 minutes, 35 cycles of 95 ℃ for 30 seconds, 58.6 ℃ for 40 seconds, 72 ℃ for 60 seconds and final extension at 72 ℃ for 5 minutes. RFLP digestion was performed in a 25 ml reaction mixture containing 5 U of MnII (New England Biolabs, USA) incubated at 37 ℃ for 16-18 hours followed by 2% agarose gel electrophoresis. The undigested PCR product with 134 bp represented T allele. The presence of G allele was confirmed by visualizing two fragments of digested PCR product with 101 bp and 33 bp.

Statistical analysis

Pearson's goodness of fit test was used to check the Hardy-Weinberg equilibrium. SPSS 22.0 (SPSS, Chicago, USA) software was used to analyze data statistically. The odds ratio (OR) and 95% confidence interval (CI) were determined to evaluate case-control study associations. Chi square test or Fisher's exact test were used to compare genotype frequencies between groups. *P*-values lower than 0.05 were considered as statistically significant.

Results

IL-10 -1082 (G/A) alleles and genotypes are associated with the increased risk of RA

The distribution of genotypes and alleles under different inheritance models (Co-dominant, Dominant, Recessive and Over-dominant) in RA patients and normal subjects were assessed and confirmed to be in Hardy–Weinberg equilibrium (HWE). Comparison of allele frequencies in patients and healthy subjects showed a significant association of G allele with RA susceptibility [OR = 1.88, 95% CI (1.32–2.66), P-value = 0.0001]. Comparison of the IL-10 -1082 (G/A) genotypes showed that the AA genotype was more frequently observed in healthy subjects. Setting AA genotype as the reference, the AG [OR = 2.93, 95% CI (1.68–5.12), P-value = 0.0001] and GG [OR = 5.73, 95% CI (2.30–14.23), P-value = 0.0001] genotypes were significantly associated with the increased risk of RA under co-dominant model. These findings were confirmed under dominant model (AA vs AG+GG) [OR = 2.88, 95% CI (1.73–4.79), P-value = 0.0001]. The over-dominant results showed that AG genotype was significantly associated with the risk of RA compared to AA+AG genotypes as reference [OR = 2.22, 95% CI (1.32–3.71), P-value = 0.0022] (Table 1).

Table1. The genotype and allele frequencies of IL-10 -1082 (G/A) rs1800896 in RA patients and healthy subjects under different inheritance models

	Genotypes and alleles	Healthy subjects (n=130) Number (%)	RA patients (n=128) Number (%)	OR (95% CI)	P-value
rs1800896	A	333 (65%)	189 (73%)	Reference	
	G	183 (35%)	71 (27%)	**1.88 (1.32-2.66)**	*0.0001*
	Co-dominant model				
	AA	76 (58.5%)	42 (32.8%)	Reference	
	AG	37 (28.5%)	60 (46.9%)	**2.93 (1.68-5.12)**	
	GG	17 (13.1%)	26 (20.3%)	**5.73 (2.30-14.23)**	*0.0001*
	Dominant model				
	AA	76 (58.5%)	42 (32.8%)	Reference	
	AG+GG	54 (41.5%)	86 (67.2%)	**2.88 (1.73-4.79)**	*0.0001*
	Recessive model				
	AA+AG	113 (86.9%)	102 (79.7%)	Reference	
	GG	17 (13.1%)	26 (20.3%)	1.69 (0.87-3.30)	*0.12*
	Over-dominant model				
	AA+GG	93 (71.5%)	68 (53.1%)	Reference	
	AG	37 (28.5%)	60 (46.9%)	**2.22 (1.32-3.71)**	*0.0022*
	X^2 HWE* (*P*-value)	2.14 *(0.20)*	0.95 *(0.47)*		

*P-values lower than 0.05 are considered as statistically significant. Significant associations are also shown in Bold. Sex and age adjustment was performed to standardize the risk assessment. Exact test for *Hardy-Weinberg equilibrium* was also conducted.

Table2. Clinical and laboratory characteristics of RA patients regarding IL-10 -1082 (G/A) genotypes

Charactristics and genotypes (N=130)		**AA**	**AG**	**GG**	*P-value*
Age		45.29±1.29	49.63±1.67	47.91±2.29	0.088
DAS28a		2.17±0.35	2.81±0.36	1.74±0.46	0.256
ESR		26.14±3.35	24.14±5.41	25.62±3.55	0.981
Age of onset		43.91±3.28	44.30±2.61*	32.00±3.29*	**0.046**
Anti-CCP	*Positive*	9.27%	9.37%	8.33%	0.988
	Negative	90.73%	90.63%	91.67%	
Family history	*Yes*	34.62%	44.44%	36.36%	0.715
	No	65.38%	55.56%	63.64%	

Data are presented as means±SE (standard error) for continuous variables and percentages of positive patients (%) for categorical variables. ESR: erythrocyte sedimentation rate; DAS28a: disease activity score 28a; Anti-ccp: anti-cyclic citrullinated peptide. Significant associations are also shown in Bold. * *P*-value <0.05

IL-10 -1082 (G/A) genotypes are not associated with clinical findings

We evaluated the association of IL-10 -1082 (G/A) genotypes with the clinical and laboratory characteristics of RA patients. Regarding the disease activity score, no significant difference was observed between all genotypes (P=0.256). No other significant association was also observed regarding the clinical and laboratory characteristics of RA patients and IL-10 -1082 genotypes (Table 2).

Discussion

Both genetic and environmental factors could be involved in the etiology and pathogenies of RA (4,5). Several risk alleles at a number of genetic loci have been introduced as susceptibility genes to RA including genetic variants in immunoregulatory cytokines (29). Several studies have investigated the association of the genetic variants in cytokines including TNF-α, TGF-β, IL-6, IFN-γ and IL-10 with RA [15, 19-21, 26, 27]. IL-10 is an anti-inflammatory cytokines which could be associated with the increased susceptibility to RA (30). SNPs in the promoter regions of IL-10 may alter the immune response to inflammatory conditions and lead to persistent inflammation (18). According to the controversies on the results of association studies between IL-10 gene promoter polymorphisms including -1082 (G/A) SNP and RA susceptibility in different regions and ethnic groups and lack of reliable studies in Iran, we aimed to assess the association of -1082 (G/A) (rs1800896) genetic variants with the susceptibility to RA in an Iranian population in comparison to normal subjects in northeast of Iran.

The first report on the association of IL-10 gene polymorphisms including -1082 (G/A)

and increased risk of RA was presented by Coakley et al in United Kingdom (17). However, no significant association was observed between IL-10 -1082 (G/A) genetic variants and RA in this study. In the present study, we showed that G allele could be introduced as a risk allele for RA development. Moreover, the AG and GG genotypes were associated with the increased risk of RA. Cantagrel et al. evaluated the association of IL-10 gene promoter SNPs with RA in a French population and showed a significant association between -1082 (G/A) alleles and genotypes with increased risk of the disease (16). Similarly, Martinez et al. revealed a significant association between -1082 (G/A) genetic variants and RA susceptibility in Spain (30). Two controversial studies in Sweden (26) and Turkey (15) reported that the AA genotype and A allele could be associated with the elevated risk of RA which were in contrast to our findings. On the other hand, Pawlik et al showed a significant association between GG genotype of -1082 (G/A) position and RA susceptibility in Poland (27) which was in accordance to our results. However, no significant association was reported between -1082 (G/A) variants and RA by Moreno et al in Columbia (22), Hee et al in Malaysia (31) Gambhir et al in India (18) (32) and Menegatti et al in Italy. Similar to our findings, De Paz et al revealed that the AA allele was more frequently observed in normal subjects and could be introduced as a protective genotype (33).

In a recent meta-analysis of 16 studies involving 2647 RA cases and 3383 healthy donors, a significant association between IL-10 -1082 (G/A) genetic variants and RA was observed for Asian and European populations by Lee et al (25). The controversial findings

in various studies might be due to the heterogeneity of populations and complicating environmental factors. Moreover, despite several association studies on IL-10 gene promoter polymorphisms and RA susceptibility worldwide, there is still lack of information in Iran.

Conclusion

Our findings revealed that IL-10 -1082 (G/A) genetic variants (GG and AG) and G allele could be associated with the increased susceptibility to RA and not with the disease activity in a population in Gorgan, northeast of Iran. According to the lack of previous reports about the association of IL-10 -1082 (G/A) and RA in Iran, the differences in allele and genotype frequencies and associations may be due to ethnic variations with other geographic regions. Although the present study contributes to better comprehend the genetic susceptibility of RA, further clinical and molecular studies are needed to elucidate the role of IL-10 gene promoter polymorphisms in RA pathogenesis.

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Conflict of interest

We declare that we have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in the article.

Authors' contributions
All authors contributed equally to this work.

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