Original Article

DOI: 10.4274/haseki.galenos.2021.6729
Med Bull Haseki 2021;59:245-248

Investigation of the Relationship Between IL-1Ra VNTR Variants and Psoriasis

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Abstract

Aim: Psoriasis is an inflammatory skin disease characterized by hyperproliferation of keratinocytes. The imbalance between Interleukin-1 (IL-1)/IL-1 receptor antagonist (IL-1Ra) is associated with increased pro-inflammatory cytokine production and the development of inflammatory disorders. The number of repeats in the polymorphic site where IL-1Ra has an 86 bp sequential repeat sequence is of functional importance as it includes possible binding sites for transcription factors. In this context, we aimed to investigate the relationship between psoriasis and IL-1Ra VNTR in Turkish society.

Methods: One hundred twenty one patients (79 female/42 male), 250 controls (142 women/108 male) were included in the study groups and genotyped by polymerase chain reaction-restriction fragment length polymorphism method. Genotype and allele distributions were examined between the patient and control groups. The gene counting method was used to calculate the allele frequencies

Results: The A1 allele is excessive in the control group (p=0.001). In addition, the fact that all of our patients with psoriasis area and severity index <=10 were carriers of the A1 allele.

Conclusions: The A1 allele may have a protective effect in terms of the severity of the disease and the determination of IL-1 variants may be a guide in determining the treatment protocols of these patients.

Keywords: Interleukin-1, interleukin 1 receptor antagonist, single nucleotide polymorphism, psoriasis

Introduction

Psoriasis is an inflammatory skin disease that affects the skin, joints and tendons, characterized by keratinocyte hyperproliferation caused by T-cell mediated genetic with environmental factors (1-3). Although the altered production of inflammatory markers is assumed to play a key role in pathogenesis, the true scenario of psoriasis etiology is still unknown (4). The severity and progression of the disease varies in different patients. In different studies attending that these individual differences may arise from genetic susceptibility (5). Currently, more than 40 independent loci may play a predisposition role in psoriasis. Interleukin-1 receptor antagonist (IL-1Ra), an anti-inflammatory cytokine and is a member of IL-1 containing three related genes (IL-1α, IL-1β and IL-1RN) within a region of 430 kb on chromosome 2q14 (6). IL-1Ra which is binding to the IL-1 receptor and competitively blocks the effects of IL-1α and IL-1β. An imbalance between IL-1 and IL-1Ra is caused to the increasing pro-inflammatory cytokine production and the development of inflammatory disorders. The IL-1Ra gene has a penta-allelic polymorphic region which is containing variable numbers of a sequential repeat sequence of 86 bp in intron two (7). The number of repeats in the polymorphic area can be of important because of repeating sequence contains possible binding sites for transcription factors. There are various conflicting data according the functional effect of these polymorphisms on IL-1α production. According to in vitro studies, the IL-1Ra A2 allele has been associated with increased IL-1Ra production in normal monocytes (8).

Several studies in various diseases including psoriasis, have shown a significant increase in the frequency of

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Received: 18.11.2020 **Accepted:** 15.02.2021

IL-1Ra A2 (9). IL-1Ra deficient mice have shown similar histologically findings in human psoriasis (10,11). In addition, it has been reported increased IL-1Ra mRNA levels in cell cultures obtained from psoriasis patients and with this information relationship between the IL-1 gene complex and psoriasis is getting important so the researchers have focused their attention on the variations in this complex (12). Polymorphisms in IL-1 gene complex are of considerable interest as they are thought to affect levels of IL-1 secretion.

However, studies which are investigating the relationship between IL-1Ra variable number of sequential repeats (VNTR) and psoriasis are quite limited and their results are conflicting. We aimed to investigate the relationship between psoriasis and IL-1Ra VNTR in Turkish population in this study.

Methods

Study Design

The present study protocol was approved by the Ethics Committee of the Giresun University Faculty of Medicine (number: KAEK-115) and written informed consents were obtained from all patients. All procedures performed in this study were in compliance with the ethical standards of the institutional and/or national research committee and the Declaration of Helsinki (13). In this study, 121 psoriasis patients who applied to Giresun University A. Ilhan Ozdemir Training and Research Hospital between 2019-2020, diagnosed clinically or histopathologically, were included in our study group. As the control group, 250 individuals who applied to the dermatology outpatient clinic with a dermatological disease and were age-matched were included in the study group. These individuals had no history of psoriasis or any chronic inflammatory disease. The demographic and clinical characteristics of both psoriasis patients and the control group were recorded in synchronized Excel files for later use. Psoriatic arthritis was investigated in all patients. Children (age <18) (n=5), pregnant and lactating participants (n=2), and participants (n=10) with a history of any immunological or inflammatory disease were excluded from the study.

Interleukin-1 Receptor Antagonist Genotype Analysis

We isolated genomic DNA from the leukocytes using Roche isolation kit (Roche high pure isolation kit, Germany). The extracted DNA was maintained at 4 °C until analysis. The VNTR mutation in the IL-1Ra gene was determined by polymerase chain reaction (PCR). The PCR product was generated by the use of oligonukleotid F: 5 "-CTCAGCAACACTCCTAT-3" R: 5 "-TCCTGGTCTGCAGGTAA-3". PCR protocol for the polymorphic region within intron 2 of the IL-1Ra gene with a VNTR of 86 bp; initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 45 s, 55 °C for 45 s and 72 °C for 45 s before terminal elongation at 72 °C for 10 min. The obtained PCR products were genotyped by imaging under UV in a 2% agarose gel and five alleles were characterized according to the number of repeats (14). Allele 1 (four repeats) was 410 bp, allele 2 (two repeats) 240 bp, allele 3 (five repeats) 500 bp, allele 4 (three repeats) was 325 bp, and allele 5 (six repeats) was 595 bp.

Statistical Analysis

The statistical analysis of this study was made using the SPSS 20 package program. Statistical significance was taken as $p<0.05$. Alleles and genotype frequencies were calculated by direct counting. The chi-square (χ^2) test was used to evaluate the intergroup differences in the frequency of genotype and alleles. Odds ratio (OR) and 95% confidence interval (95% CI) are given to determine the risk factor between groups.

Results

The demographic characteristics of the study groups are given in Table 1. The patients were divided into two groups according to the age of onset of psoriasis [<=40: early onset (n=78); >40: late onset (n=43)]. Among our patients, 92 people had no psoriatic arthritis, while 29 suffered from psoriatic arthritis. In addition, 101 patients did not have a family history of psoriasis, while 20 patients had a history of psoriasis in their first degree relatives.

In the patient and control groups included in the study, only 3 alleles [A1=410 (four replicates), A2=242 (two replicates), A3=500 (five replicates)] were observed (Table

Table 1. Demographical characteristics of the study population

		Cases (n=121)	Controls (n=250)	p
Age (Years)	(Mean ± SD)	44.08±14.17	47.61±12.0	NS
Sex n (%)				
Male Female	-	42 (34.7%) 79 (65.3%)	108 (43.2%) 142 (56.8%)	NS
Artrit n (%)				
Artrit + Artrit -	-	29 (24.0%) 92 (76.0%)	- -	-
Age of onset n (%)	<=40 >40	78 (65.0%) 43 (35.0%)	-	-
Family history (AÖ) n (%)	AÖ+ AÖ-	20 (16.5%) 101 (83.4%)	-	-
PASI n (%)	>10 <=10	108 (89.3%) 13 (10.7%)	-	-

Mean values were compared between patients and controls by using the Student's t-test. Qualitative data were analyzed by the chi-square test. NS: Not significant, PASI: Psoriasis area and severity index, Data are presented as mean ± SD and n (%). Bold values were statistically significant ($p<0.05$). n: Number of samples

2). The frequency distribution of these alleles in Turks is similar to other world populations, A1 is the most common allele, followed by A2 and A3 are rare alleles. There was a statistically significant difference in genotype and allele frequencies between patients and controls (p<0.001). The IL-Ra VNTR allele and genotype frequencies in psoriasis patients and controls are shown in Table 2. The A1 allele was present at a significantly higher frequency in the control group (p=0.001, OR: 0.322% 95 CI: 0.159-0.655). In addition, interestingly, all of our patients with Psoriasis area and severity index (PASI) <=10 are carriers of the A1 allele (data not shown). The A2 and A3 allele did not show a significant difference between the two groups (p=0.363; p=0.560, respectively).

Discussion

Two important studies in 2014 and 2019 highlighted the importance of the IL-1β-IL-1R signaling pathway in psoriasis. In one of this studies Lowes et al. (3) showed IL-1β-IL-1R signaling pathway plays critical roles in psoriasis pathogenesis and in the other Cai et al. (15) data showed that the IL-1β-IL-1R signaling pathway is associated with disease progression and treatment response. The results of these two studies suggest that this pathway not only a target for the treatment of the disease,it also for a important target in disease progression and response of treatment.

Di Paolo et al. (16) demonstrated the effects of IL-1Ra on blocking IL-1α and IL-1β activity. In addition, IL-1Ra knockout mice developed skin inflammation with histopathological characteristics similar to human psoriasis (17), while positive effects were observed in individuals with psoriasis treated with recombinant IL-1Ra (18). The significantly higher mRNA expressions of IL-1 receptor antagonists evaluated in peripheral blood mononuclear cells (PBMCs) of 10 psoriatic patients and six healthy controls in psoriasis patients also a documentation of the relationship between the IL-1 family and psoriasis (12). When this information is evaluated and the role of IL-1Ra in controlling inflammation is considered, it can be assumed that the variations in this gene may contribute to the pathogenesis of psoriasis by affecting gene expression levels. Studies showing that the IL-1Ra VNTR A2 allele is associated with a variety of epithelial-related chronic inflammatory diseases, including alopecia areata, lichen sclerosis, systemic lupus erythematosus, ulcerative colitis and scleroderma (19,20). A few studies which are investigating the relationship between psoriasis and IL-1Ra, are very limited and contains conflicting results.

One of study from the G.Britain by Tarlow et al. (9) showed that the frequency of the A2 allele increased in the early-onset (<40 years) cohort with psoriasis and decreased significantly in the late-onset (>40 years) cohort compared to controls, and on the other hand Moorchung et al. (21) reported that there is no relationship between VNTR and psoriasis.

In addition; other studies from Egypt, Taiwan, and Canadia showed that there was no significant difference in the frequencies of all genotypes and alleles related to the IL-1Ra VNTR polymorphism (22-24). Finally, a meta analysis result emphasized that there is no relationship between IL-1Ra and Psoriasis pathogenesis (25).

In the results of our study, the A1 allele was found to be statistically significantly higher in the control group compared to the patient group. This suggests that the A1 allele may have a protective effect against the severity of the disease. In addition, the fact that all of our patients with PASI <=10 were carriers of the A1 allele also supports this finding. A2 and A3 alleles did not show statistically significant differences in patient and control groups, consistent with the studies from Egypt, Taiwan, and Canadia.

Table 2. Genotype and allele distributions in study groups

Genotip								
	A1/A1		**A1/A2**	**A1/A3**		**A2/A2**		**A3/A3**
Cases n (%) (n=121)	50 (41.3%)		42 (34.7%)	7 (5.8%)		10 (8.3%)		10 (8.3%)
Controls n (%) (n=250)	117 (46.8%)		109 (43.6%)	11 (4.4%)		13 (5.2%)		0 (0.0%)
p	<0.001*							
Allele								
	A1 (+)	A1 (-)	A2 (+)	A2 (-)	A3 (+)	A3 (-)	A4 (+)	A5 (+)
Cases (n=121)	0.83	0.16	0.43	0.57	0.05	0.95	0.0	0.0
Controls (n=250)	0.94	0.06	0.48	0.52	0.04	0.96	0.0	0.0
X^2	10.579		0.828		0.339		-	-
p	0.001**		0.8363		0.560		-	-

Data are presented as n (%). Bold values were statistically significant (p<0.05). n: Number of samples
*: X^2 test (two degrees of freedom)
**: X^2 test (one degree of freedom)

Study Limitations

Our study has some limitations because of IL-1Ra serum levels and expression levels could not compared. Therefore, we think that our study should be supported by new studies which have expression levels in a larger sample group.

Conclusion

Our study is the first study which was investigating psoriasis and IL-1Ra VNTR polymorphism in Turkish population. As a result; we think that the A1 allele may have a protective effect in terms of the severity of the disease and the determination of IL-1Ra variants may be a guide in determining the treatment protocols for these patients.

Authorship Contributions

Concept: A.B.A.T., Design: A.B.A.T., B.A., Data Collection or Processing: A.B.A.T., B.A., Analysis or Interpretation: A.B.A.T., B.A., Literature Search: A.B.A.T., B.A., Writing: A.B.A.T.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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