EJR European Journal of Rheumatology

DOI: 10.5152/eurjrheum.2017.16102

Original Article

Investigation of the role of interleukin-1 receptor antagonist VNTR variant on the Behçet's disease

Gül Dursun[1], Ayşe Feyda Nursal[2], Helin Deniz Demir[3], Nevin Karakuş[1], Osman Demir[4], Serbülent Yiğit[1]

Abstract

Objective: Behçet's disease (BD), a chronic multisystem inflammatory disorder, is mainly characterized by relapsing periods of a wide range of clinical symptoms. Several cytokine genes may play important roles in the pathogenesis of BD. Therefore, interleukin-1 receptor antagonist (IL-1Ra) gene 86bp variable number tandem repeat (VNTR) variant was investigated in patients with BD in a Turkish population.

Methods: One hundred nine patients (60 females, 49 males; the mean age±standard deviation [SD] was 36.56±9.571 years) with BD and one hundred healthy individuals (54 females, 46 males; the mean age±SD was 36.64±2.294 years) were examined in the study. For genotyping, polymerase chain reaction-restriction fragment length polymorphism analysis was employed. Data were analyzed using Statistical Package for Social Sciences (SPSS) 22.0 (IBM Corp.; Armonk, NY, USA) ($p<0.05$)

Results: The genotype distribution and allele frequencies of the IL-1Ra VNTR variant did not differ significantly between the patients and the controls ($p>0.05$). The frequency of the a1/a1, a1/a2 genotypes and a1, a2 alleles were the most common both in patients and healthy controls ($p=0.37$, $p=0.26$, and $p=0.53$, respectively). Also, no statistically significant difference was found between the IL-1Ra VNTR variant genotypes and clinical characteristics ($p>0.05$).

Conclusion: The results of this study do not support an association between the IL-1Ra VNTR variant and the risk of BD in a Turkish population. However, further studies of this variant with larger sample sizes and different ethnicities are required for confirmation.

Keywords: Behçet's disease, interleukin 1 receptor antagonist, variable number tandem repeat

ORCID ID's of the authors:
G.D. 0000-0001-5125-5941;
A.F.N. 0000-0001-7639-1122;
H.D.D. 0000-0003-4396-0872;
N.K. 0000-0002-1916-7471;
O.D. 0000-0002-1322-2716;
S.B. 0000-0002-1019-3964.

Cite this article as: Dursun G, Nursal AF, Deniz Demir H, Karakuş N, Demir O, Yiğit S. Investigation of the role of interleukin-1 receptor antagonist VNTR variant on the Behçet's disease. Eur J Rheumatol 2018; 5: 27-31.

[1] Department of Medical Biology, Gaziosmanpaşa University School of Medicine, Tokat, Turkey
[2] Department of Medical Genetics, Hitit University School of Medicine, Çorum, Turkey
[3] Department of Opthalmology, Gaziosmanpaşa University School of Medicine, Tokat, Turkey
[4] Department of Biostatistics, Gaziosmanpaşa University School of Medicine, Tokat, Turkey

Address for Correspondence: Ayşe Feyda Nursal, Department of Medical Genetics, Hitit University School of Medicine, Çorum, Turkey

E-mail: feydanursal@hotmail.com, feyda.nursal@gmail.com

Submitted: 22 November 2016
Accepted: 12 April 2017
Available Online Date: 25 October 2017

Introduction

Behçet's disease (BD; MIM 109650) is a chronic disease manifested by multisystem involvement including oral and genital ulcers, skin lesions, uveitis, arthritis, and neurological symptoms. The average age of onset is during the early 30s, and the male to female ratio differs depending on ethnicity (1). BD is more prevalent in the Far East, the Mediterranean, and the Middle East (2). The prevalence is about 0.64/100,000 in the UK, 5.2 in the USA, and as high as 421 in Turkey (3). The precise pathogenesis of BD is controversial; however, genetic, immunological, and environmental factors have been offered to account for the development of BD (4). The human leukocyte antigen B51 gene is found to be the most reliable marker for this disorder in several ethnic populations, but it is only partially responsible for the genetic tendency to BD (5).

Inflammation is a characteristic feature of BD, and it is believed to be related to cytokines (6). Interleukin-1 (IL-1) is a pro-inflammatory cytokine and main mediator of immune responses. IL-1 is produced in two distinct forms: IL-1A and IL-1B. They bind to the IL-1 receptor. The IL-1 receptor antagonist (IL-1Ra) hinders the IL-1-mediated inflammation response by inhibiting the affinity of IL-1 for the IL-1 type-I receptor. Human IL-1A, IL-1B, and IL-1Ra genes form a cluster on chromosome 2q12 to 2q14 (7). In intron 2 of the IL-1Ra gene, a variant associated with the varying numbers of an 86bp tandem repeat (VNTR) (rs2234663) was identified (8). This variant leads to the existence of five alleles resulting from a variable number of repeats (8). The number of repeats has functional significance. Therefore, a balance between IL-1 and IL-1Ra is an essential factor in normal and disease conditions that determines the degree of the inflammatory response to an environmental stimulus that is impaired due to the IL-1Ra VNTR variant (9).

The IL-1Ra VNTR variant has been associated with severity of or susceptibility to several inflammatory disorders.

Therefore, the aim of this study was to investigate whether there is a relationship between IL-1Ra VNTR variant and BD risk in a Turkish cohort.

Methods

Study population

The study population consisted of 109 unrelated patients with BD (60 females, 49 males) attending the Opthalmology Department of Gaziosmanpaşa University Research Hospital, Tokat, Turkey. A total of 100 individuals (54 females, 46 males) matched by ethnicity, age, and sex were recruited consecutively. Subjects in this study were older than 18 years and were part of Turkish population from the central Black Sea region of Turkey. Detailed clinical characteristics were recorded for each patient. Patients were diagnosed according to the International Criteria for BD (10). All patients were informed of the study protocol, and their written informed consent was acquired. The protocol of the study was approved by the local ethics committee, and the study was carried out in accordance with the Declaration of Helsinki.

Genotyping of IL-1Ra VNTR variant

Genomic DNA was isolated from 2 mL of venous blood using a commercial DNA isolation kit (SigmaAldrich, St. Louis, MI USA) and stored at 20°C until the time of use. The 86bp VNTR variant of IL-1Ra was analyzed according to the protocol described previously (8). Polymerase chain reaction (PCR) was performed in a 25 µL final volume containing 25 pM of each primer, 0.1 mM of dNTP, 0.5 µg of genomic DNA, 1. 5 mM of MgCl, 2 and 2.5 µL of PCR buffer, and 1.5 unit of Taq DNA polymerase according to the following protocol: initial denaturation at 94°C for 4 min; 30 cycles of denaturation at 94°C for 45 s, annealing at 51°C for 30 s, and extension at 72°C for 45 s; and final extension at 72°C for 5 min. Two oligonucleotide primers, 5'CTC AGC AAC ACT CCT AT 3' (forward) and 5' TTC CAC CAC ATG GAA C 3' (reverse) based on the flanking region of the IL-1Ra gene were utilized. PCR products were separated by electrophoresis on a 3% agarose gel and visualized after ethidium bromide staining. Five different alleles of IL-1Ra were described as follows: allele 1, four repeats (410 bp); allele 2, two repeats (240 bp); allele 3, five repeats (500 bp); allele 4, three repeats (325 bp); and allele 5, six repeats (595 bp).

Statistical analysis

All statistical analyses were done using Statistical Package for Social Sciences 22.0 (IBM Corp.; Armonk, NY, USA) and OpenEpi info 2.2 statistical software. Continuous data were given as mean±SD and min/max. The chi-square test was used to detect the significance of differences in the allele frequency and genotype distribution between the two study groups. The normality of assumption was assessed with the Kolmogrov-Smirnov or Shapiro-Wilk tests. The Hardy-Weinberg equilibrium (HWE) test was performed for both study groups. An odds ratio (OR) and 95% confidence intervals (CIs) were calculated. A $p<0.05$ was considered statistically significant.

Results

In the present study, a total of 209 subjects, including 109 BD patients and 100 adult healthy controls, were genotyped for the IL-1Ra VNTR variant. Baseline demographic characteristics of the patients and controls are shown in Table 1. Regression analysis was performed. The results of regression analysis are listed in Table 2. The mean age±SD was 36.56±9.571 years in patients and 36.64±2.294 years in the control group (p=0.935). There were 60 (55%) women and 49(45%) men in the patient group; in control group, there were 54 (54%) women and 46 (46%) men.

Genotype and allele frequencies of the analyzed samples of the IL-1Ra VNTR variant are shown in Table 3. The frequency of a1/a1 and a1/a2 genotypes and alleles a1, a2 were the most common in both patient group and healthy control (p=0.37). The most frequently observed genotype was a1/a1 (51.3%) followed by a1/a2 (37.6%) in the patient group. The overall distribution of IL-1Ra genotypes did not show any significant difference between BD patients and the controls. Although the difference was not statistically significant, the a2/a2 genotype was lower in the patient group than in the controls.

Five alleles were seen in patients and control subjects. In this study, we detected the following IL-1Ra alleles: a1 (70.1%) (p=0.26, OR:1.267 95%CI: 0.831.91) and a2 (23.3%) (p=0.53, OR:0.869 95%CI: 0.5551.359) in the patients and a1 (65%) and a2 (26%) in the controls. No significant difference was observed in the frequency of IL-1Ra alleles between the patients and the control group. Other alleles were 6.4%

Table 1. The demographic characteristics of patients with BD and healthy controls

	Patients (n=109) (%)	Controls (n=100) (%)	p
Age, mean±SD (years)	36.56±9.571	36.64±2.294	0.935
Gender, n (%)			
Male	49 (45.0)	46 (46.0)	
Female	60 (55.0)	54 (54.0)	0.879

Table 2. Result of logistic regression

					95% C.I.for odds ratio	
	β	SE	p	Odd ratio	Lower	Upper
Gender	-0.047	0.282	0.866	0.954	0.548	1.658
Age	-0.002	0.02	0.913	0.998	0.96	1.038

Reference category for gender is female. SE: standard error; β: regression coefficient

Table 3. Genotype and allele frequencies of the VNTR variant of IL-1Ra in the groups

	Patients n=109 (%)	Controls n=100 (%)	p	OR (95% CI)
Genotype				
a1/a1	56 (51.3)	49 (49)	0.37	
a1/a2	41 (37.6)	32 (32)		
a2/a2	5 (4.58)	10 (10)		
Others:(a1/a3, a1/a4, a1/a5, a2/a4, a4/a5)	7 (6.42)	9 (9)		
Allele				
a1	153 (70.1)	130 (65)	0.26	1.267 (0.83-1.91)
a2	51 (23.3)	52 (26)	0.53	0.869 (0.555-1.359)
Others:(a3, a4, a5)	14 (6.4)	18 (9)	0.33	0.694 (0.329-1.443)

Table 4. Clinical and demographic characteristics of patients and controls according to IL-1Ra VNTR genotypes

		IL-1Ra gene VNTR variant				
Characteristics	Total (n=109)	a1/a1	a1/a2	a2/a2	Others	p
Age, mean ± SD(years)	36.56±9.57	36.95±9.31	36.32±9.88	36.60±8.56	34.86±12.22	0.95
Gender, n (%)						
Female	60 (55.0)	32 (57.1)	21 (51.2)	21 (40.0)	5 (71.4)	0.66
Male	49 (45.0)	33 (78.6)	20(48.8)	20(60.0)	2 (28.6)	
Clinical findings n (%)						
Genital ulcers						
Yes	94 (86.2)	48 (85.7)	37 (90.2)	3 (60.0)	6 (85.7)	0.32
No	15 (13.8)	8 (14.3)	4 (9.8)	2 (40.0)	1 (14.3)	
Papulopustular lesion						
Yes	78 (71.6)	39 (69.6)	31 (75.6)	4 (80.0)	4 (57.1)	0.72
No	31 (28.4)	17 (30.4)	10 (24.4)	1 (20.0)	3 (42.9)	
Erythema nodosum						
Yes	66 (60.6)	33 (58.9)	24 (58.5)	3 (60.0)	6 (85.7)	0.57
No	43 (39.4)	23 (41.1)	17 (41.5)	2 (40.0)	1 (2.3)	
Ocular involvement						
Yes	100 (91.7)	50 (98.3)	38 (92.7)	5 (100.0)	7 (100.0)	0.66
No	9 (8.3)	6 (52.4)	3 (7.3)	0 (0)	0 (0)	
Skin involvement						
Yes	83 (76.1)	44 (78.6)	27 (65.9)	5 (100.0)	7 (100.0)	0.09
No	26 (23.9)	12 (10.7)	14 (34.1)	0 (0)	0 (0)	
Disease duration Mean±SD (range) years	7.38±6.54	7.44±5.88	6.63±5.29	9.20±9.47	10.07±13.86	0.55
Treatment duration Mean±SD (range) years	6.16±6.98	5.91±5.96	5.21±4.85	7.80±10.28	9.78±14.05	0.45
Colchicine treatment n (%)						
500 mg	5 (100.0)	2 (40.0)	2 (40.0)	1 (20.0)	0 (0)	0.68
1000 mg	18 (100.0)	9 (50.0)	5 (27.8)	2 (11.1)	2 (11.1)	
1500 mg	43 (100.0)	22 (51.2)	15 (34.9)	1 (2.3)	5 (11.6)	
1000/1500 mg	16 (100.0)	10 (62.5)	5 (31.3)	1 (6.3)	0 (0)	
Response to treatment n (%)						
Yes	53 (67.1)	26 (63.4)	16 (61.5)	5 (100)	6 (85.7)	0.24
No	26 (32.9)	15(36.6)	10(38.5)	0 (0)	1 (14.3)	

in patients and 9% in the controls (p=0.33, OR: 0.694 95%CI: 0.3291.443).

Furthermore, we also analyzed if any differences existed in the clinical and demographic characteristics of patients according to genotype distribution. The clinical and demographic characteristics according to genotype distribution are presented in Table 4. Post-power analysis was 49% for the skin involvement variable. There was no significant difference between the IL-1Ra VNTR variant genotypes and the clinical characteristics (p>0.05).

Discussion

Behçet's Disease is a multifactorial disease with a genetic background which, along with environmental risk factors including infectious agents, is likely to be significant in establishing the susceptibility. Despite the extensive researches for identifying the genetic basis for this disease, there is still limited amount of knowledge on this topic. Therefore, several distinct genes are being tested in terms of relation with BD. It was found that some cytokine variants such as IL-27, IL-23, and IL-10 are associated with BD in different populations (11-14).

The IL-1 superfamily contains some of the essential cytokines in the host-pathogen immune response (15). Although IL-1α and IL-1β have strong pro-inflammatory activity, the

antagonist of IL-1Ra interferes with the transmission of pro-inflammatory signals, hindering the immune response (16). Numerous studies have found that IL-1Ra levels are useful in predicting the host's immune response, because the values are elevated during the final stages of inflammatory response. Most of the studies about the relationship of IL-1Ra gene variants with disease susceptibility were conducted on patients who had autoimmune diseases or conditions associated with chronic inflammation.

Intron 2 of the IL-1Ra gene has a 86bp VNTR variant that bears three potential protein-binding sites: an interferon α silencer A, an interferon β silencer B, and an acute phase response element. It has been suggested that cell proliferation activity is regulated by IL-1Ra production through these three binding sites (8). The IL-1Ra variant has been reported to be related to several diseases including systemic lupus erythematosus, alopecia areata, lichen sclerosus, and ulcerative colitis (17-20).

The frequency of the alleles differs among various ethnic or geographic groups. The four repeats (allele 1) and two repeats (allele 2) variants are the most common, while the other alleles are seldom (<5%) (17). Of these five alleles, allele 2 plays an important role in the molecular basis of various diseases and autoimmune disorders. It was reported that IL-1Ra allele 2 was linked with higher plasma levels of IL-1Ra than the non-carriers (16). Furthermore, it was reported that IL-1Ra allele 2 was related to less production of IL-1α protein and increased production of IL-1β by monocytes in vitro (21). It was suggested that carriers of genotype IL-1Ra a2/a2 have a more prolonged and more severe proinflammatory immune response. This, in turn, results in a further increase in IL-1Ra synthesis and a prolonged inflammatory reaction (16). In theory, elevated IL-1Ra synthesis should decrease binding to its receptors, contributing to anti-inflammatory effects. It was concluded that IL-1Ra allele2 mainly lowers the level of IL-1β and thus leads to an impairment in the IL-1β/IL-1Ra ratio, resulting in more susceptibility to severe outcome of inflammatory diseases. A possible relation between IL-1Ra allele 2 and numerous rheumatic disorders has been investigated. A relation was suggested between IL-1Ra allele 2 and severe forms of Sjogren's syndrome, skin disease in systemic lupus erythematosus, juvenile idiopathic inflammatory myopathies, and juvenile chronic arthritis, especially in patients with a prolonged oligoarticular involvement (17, 22-24).

In the present study, we investigated the impact of the IL-1Ra VNTR variant and BD in our population. In previously studies conducted on Turkish population, it was reported that the frequency of the IL-1Ra genotype and allele was not different between patients with BD and healthy controls (25-27). Also, Karesneh et al. (28) examined the potential relation between the specific variants of IL-1α, IL-1β, and IL-1Ra with susceptibility to BD in a study performed on 132 patients and 105 healthy controls, and they showed that the IL-1Ra VNTR variant is not related to BD. Our results are consistent with those found by Coskun et al. (25), Barıs et al. (26), Ozcimen et al. (27), and Karasneh et al. (28). Since the patients with BD exhibited various clinical characteristics, we further examined the association between the IL-1Ra VNTR variant and different clinical parameters such as genital ulcers, papulopustular lesions, erythema nodosum, and disease duration. No significant difference was noted between any of the above-mentioned clinical features and the IL-1Ra VNTR variant.

In conclusion, these results did not indicate any association between BD and the IL-1Ra VNTR variant in Turkish population. To clarify this issue, further studies of this variant should include larger sample sizes and different ethnicities to determine its effect on BD risk.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziosmanpaşa University 15-KAEK-142.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - G.D., S.Y., N.K.; Design - G.D., N.K., A.F.N.; Supervision - A.F.N., H.D.D., S.Y.; Resources - H.D.D., O.D.; Materials - A.F.N., G.D., N.K.; Data Collection and/or Processing - H.D.D., N.K., O.D.; Analysis and/or Interpretation - G.D., S.Y., N.K., O.D.; Literature Search - N.K., A.F.N., S.Y.; Writing Manuscript - A.F.N., S.Y.; Critical Review - N.K., S.Y.

Acknowledgements: This study was supported by the Gaziosmanpaşa University Research Fund and is a report of Master of Science thesis.

Conflict of Interest: The authors declared no conflict of interest .

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

References

1. Akyol L, Gunbey E, Karlı R, Onem S, Ozgen M, Sayarlıoglu M. Evaluation of olfactory function in Behçet's disease. Eur J Rheumatol 2016; 3: 153-6. [CrossRef]
2. Kapsimali VD, Kanakis MA, Vaiopoulos GA, Kaklamanis PG. Etiopathogenesis of Behcet's disease with emphasis on the role of immunological aberrations. Clin Rheumatol 2010; 29: 1211-6. [CrossRef]
3. Mohammad A, Mandl T, Sturfelt G, Segelmark M. Incidence, prevalence and clinical characteristics of Behcet's disease in southern Sweden. Rheumatology (Oxford) 2013; 52: 304-10. [CrossRef]
4. Zierhut M, Mizuki N, Ohno S, Inoko H, Gul A, Onoe K, et al. Immunology and functional genomics of Behcet's disease. Cell Mol Life Sci 2003; 60: 1903-22. [CrossRef]
5. Meng Q, Guo H, Hou S, Jiang Z, Kijlstra A, Yang P. Lack of an association of PD-1 and its ligand genes with Behcet's disease in a Chinese Han population. PLoS One 2011; 6: e25345. [CrossRef]
6. Gul A. Behcet's disease: an update on the pathogenesis. Clin Exp Rheumatol 2001; 19: 6-12.
7. Nicklin MJ, Weith A, Duff GW. A physical map of the region encompassing the human interleukin-1 alpha, interleukin-1 beta, andinterleukin-1 receptor antagonist genes. Genomics 1994; 19: 382-4. [CrossRef]
8. Tarlow JK, Blakemore AI, Lennard A, Solari R, Hughes HN, Steinkasserer A, et al. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet 1993; 91: 403-4. [CrossRef]
9. Dinarello CA. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. Int Rev Immunol 1998; 16: 457-99. [CrossRef]
10. International study group for Behcet's disease. Criteria for diagnosis of Behcet's disease. Lancet 1990; 335: 1078-80.
11. Dehghanzadeh R, Babaloo Z, Sakhinia E, Khabazi A, Shanehbandi D, Sadigh-Eteghad S, et al. IL-27 Gene Polymorphisms in Iranian Patients with Behcet's Disease. Clin Lab 2016; 62: 855-61. [CrossRef]
12. Yalcin B, Atakan N, Dogan S. Association of interleukin-23 receptor gene polymorphism with Behçet disease. Clin Exp Dermatol 2014; 39: 881-7. [CrossRef]
13. Wu Z, Zheng W, Xu J, Sun F, Chen H, Li P, et al. IL10 polymorphisms associated with Behçet's disease in Chinese Han. Hum Immunol 2014; 75: 271-6. [CrossRef]
14. Hu J, Hou S, Zhu X, Fang J, Zhou Y, Liu Y, et al. Interleukin-10 gene polymorphisms are associated with Behcet's disease but not with Vogt-Koyanagi-Harada syndrome in the Chinese Han population. Mol Vis 2015; 21: 589-603.
15. Dinarello CA. Biologic basis for Interleukin-1 in disease. Blood 1996; 87: 2095-147.
16. Hurme M, Santtila S. IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1beta genes. Eur J Immunol 1998; 28: 2598-602. [CrossRef]
17. Blakemore AI, Tarlow JK, Cork MJ, Gordon C, Emery P, Duff GW. Interleukin-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. Arthritis Rheum 1994; 37: 1380-5. [CrossRef]
18. Tarlow JK, Clay FE, Cork MJ, Blakemore AI, McDonagh AJ, Messenger AG, et al. Severity of

alopecia areata is associated with a polymorphism in the interleukin-1 receptor antagonist gene. J Invest Dermatol 1994; 103: 387-90. [CrossRef]
19. Clay FE, Cork MJ, Tarlow JK, Blakemore AI, Harrington CI, Lewis F, et al. Interleukin 1 receptor antagonist gene polymorphism association with lichen sclerosus. Hum Genet 1994; 94: 407-10. [CrossRef]
20. Mansfield JC, Holden H, Tarlow JK, Di Giovine FS, McDowell TL, Wilson AG, et al. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. Gastroenterology 1994; 106: 637-42. [CrossRef]
21. Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. Scand J Immunol 1998; 47: 195-98. [CrossRef]
22. Perrier S, Coussediere C, Dubost JJ, Albuisson E, Sauvezie B. IL-1 receptor antagonist (IL-1RA) gene polymorphism in Sjogren's syndrome. Clin Immuol Immunopathol 1998; 87: 309-13. [CrossRef]
23. Rider LG, Artlett CM, Foster CB, Ahmed A, Neeman T, Chanock SJ, et al. Polymorphisms in the IL-1 receptor antagonist gene VNTR are possible risk factors for juvenile idiopathic inflammatory myopathies. Clin Exp Immunol 2000; 121: 47-52. [CrossRef]
24. Vencovsky J, Jarosova K, Ruzickova S, Němcová D, Niederlová J, Ozen S, et al. Higher frequency of allele 2 of the interleukin-1 receptor antagonist gene in patients with juvenile idiopathic arthritis. Arthritis Rheum 2001; 44: 2387-91. [CrossRef]
25. Coskun M, Bacanli A, Sallakci N, Alpsoy E, Yavuzer U, Yegin O. Specific interleukin-1 gene polymorphisms in Turkish patients with Behçet's disease. Exp Dermatol 2005; 14: 124-9. [CrossRef]
26. Barıs S, Akyürek O, Dursun A, Akyol M. The impact of the IL-1β, IL-1Ra, IL-2, IL-6 and IL-10 gene polymorphisms on the development of Behcet's disease and their association with the phenotype. Med Clin (Barc) 2016; 146: 379-83. [CrossRef]
27. Ozçimen AA, Dilek K, Bingol U, Sarıcaoğlu H, Sarandol A, Taskapılıoğlu O, et al. IL-1 cluster gene polymorphisms in Turkish patients with Behçet's disease. Int J Immunogenet 2011; 38(4): 295-301. [CrossRef]
28. Karasneh J, Hajeer AH, Barrett J, Ollier WE,Thornhill M, Gul A. Association of specific interleukin 1 gene cluster polymorphisms with increased susceptibility for Behcet's disease. Rheumatology (Oxford) 2003: 42: 860-4. [CrossRef]