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Türkiye'de Behçet Hastalarında NALP3 Q705k Gen Polimorfizmi Sıklığı

NALP3 Q705K Gene Polymorphism Frequency in Behçet's Disease Patients in Turkey

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ABSTRACT

Objectives: This study aims to investigate Q705K polymorphism in NALP3 gene in Behçet's disease (BD).

Patients and methods: This case-control study included 180 BD patients (92 males, 88 females; mean age 36.6±11.3 years; range 21 to 56 years) and 168 healthy controls (92 males, 76 females; mean age 34.9±13.2 years; range 22 to 55 years). Cellular deoxyribonucleic acid was isolated from peripheral blood using standard procedures. Patients and the controls were genotyped by polymerase chain reaction-restriction fragment length polymorphism method for NALP3 Q705K gene polymorphism. Polymorphic region was amplified by polymerase chain reaction and digested with BsgI enzyme. Genotype frequencies between the groups were compared by K2-square, Fisher's exact, and chi-square tests.

Results: All BD patients had oral ulcers. Of the patient group, genital ulcers were present in 78%, folliculitis in 66.3%, vascular involvement in 31.2%, uveitis in 66.1%, and neurological manifestations in 7.5%. Of the control group, homozygous genotype was present in 1.2% (n=2) and heterozygous genotype in 11.3% (n=19), whereas of the patient group, homozygous genotype was present in 1.1% (n=2) and heterozygous genotype in 8.3% (n=16), without a statistically significant difference between the groups. Comparison of allele frequencies between two groups did not reveal a statistically significant difference (5.5% and 6.8%, respectively). Subgroup analysis according to ocular, mucocutaneous, vascular, articular, and neurologic involvement also revealed no statistical differences.

Conclusion: According to our findings, distribution of NALP3 Q705K gene polymorphism shows no significant difference between patient and control groups in terms of disease susceptibility and system involvements in BD in Turkey.

Key words: Behçet's disease; NALP3; NLRP3 gene; Q705K gene polymorphism; single nucleotide polymorphism.

ÖZ

Amaç: Bu çalışmada, Behçet hastalığında (BH) NALP3 geninde Q705K polimorfizmi araştırıldı.

Hastalar ve yöntemler: Bu olgu-kontrol çalışmasına 180 BH hastası (92 erkek, 88 kadın; ort. yaş 36.6±11.3 yıl; dağılım 21-56 yıl) ve 168 sağlıklı kontrol (92 erkek, 76 kadın; ort. yaş 34.9±13.2 yıl; dağılım 22-55 yıl) alındı. Deoksiribonükleik asit periferik kandan standart işlemler kullanılarak elde edildi. Hastalar ve kontroller polimeraz zincir reaksiyonu-restriksiyon fragman uzunluk polimorfizmi yöntemi ile NALP3 Q705K gen polimorfizmi açısından genotiplendirildi. Polimorfik bölge polimeraz zincir reaksiyonu ile çoğaltılarak Bsg1 enzimi ile parçalandı. Gruplar arasındaki genotip sıklıkları K2-kare, Fisher kesin ve ki-kare testleri ile karşılaştırıldı.

Bulgular: Tüm BH hastalarında oral ülser vardı. Hasta grubunun %78'inde genital ülser, %66.3'ünde follikülit, %31.2'sinde damar tutulumu, %66.1'inde üveit ve %7.5'inde nörolojik görünümler vardı. Kontrol grubunun %1.2'sinde (n=2) homozigot genotip ve %11.3'ünde (n=19) heterozigot genotip var iken, hasta grubunun %1.1'inde (n=2) homozigot genotip ve %8.3'ünde (n=16) heterozigot genotip vardı; gruplar arasında istatistiksel olarak anlamlı bir farklılık yoktu. İki grup arasındaki alel frekansları karşılaştırıldığında, istatistiksel olarak anlamlı bir farklılık görülmedi (sırasıyla %5.5 ve %6.8). Oküler, mukokutan, vasküler, artiküler ve nörolojik tutulumlara göre alt grup analizi de istatistiksel olarak anlamlı bir farklılık göstermedi.

Sonuç: Bulgularımıza göre, Türkiye'de BH'de hastalığa yatkınlık ve sistem tutulumları açısından NALP3 Q705K gen polimorfizmi dağılımı hasta ve kontrol grupları arasında anlamlı bir farklılık göstermemektedir.

Anahtar sözcükler: Behçet hastalığı; NALP3; NLRP3 geni; Q705K gen polimorfizmi; tek nükleotid polimorfizmi.

Behçet's disease (BD) is an immune-mediated systemic inflammatory disorder characterized by vasculitis of especially small vessels. It has a wide range of clinical presentation consisting of recurrent oral and genital ulcers, eye and skin lesions, and systemic involvement of central nervous system, gastrointestinal and musculoskeletal system. Behçet's disease has a particular geographical distribution along the ancient route known as 'The Silk Road', stretching from China to the Mediterranean area including Turkey. The prevalence of the disease is 80-370/100,000 in Turkey.[1,2]

The etiology of BD is not well documented. Both environmental (such as infectious agents)[3,4] and genetic factors[5,6] contribute to the pathogenesis. The familial aggregation supports genetic predisposition in BD. Most widely accepted and documented genetic association is human leukocyte antigen (HLA)-B51. The frequency of HLA-B51 is as high as 50 to 80% in particular ethnic groups with BD. There are other studies reporting additional independent associations such as HLA-B*15, -B*27, -B*57, and -A*26.[7-9] Under the light of the literature, the most widely accepted hypothesis is that it is a complex disease caused by overactive acquired and innate immune response against environmental and auto-antigens in genetic predisposed individuals. Beside this, BD is not a classic autoimmune disease, but it also has common features with auto-inflammatory diseases.[10,11] Auto-inflammatory diseases are characterized by recurrent inflammatory episodes with neutrophil over-activation. Enhanced inflammatory response and over-expression of proinflammatory cytokines are also prominent features of both BD and autoinflammatory disorders.[10]

Interleukin (IL)-1β is a potent proinflammatory cytokine that is closely associated with innate immunity. It promotes inflammatory response by increasing expression of adhesion molecules and chemokine genes. Uncontrolled production of IL-1β is associated with auto-inflammatory diseases such as cryopirin associated periodic syndromes (CAPS). The common characteristic of these diseases is that IL-1β plays a central role in the pathogenesis and blockage of IL-1β production provides a remarkable improvement of symptoms.[12]

NALP3 protein (formerly known as cryopirin) encoded by NLRP3 gene is a part of inflammasome. It activates caspase 1 which in turn activates IL-1 and IL-18 from their inactive forms.[13] Gain of function mutation of the gene causes uncontrolled production of IL-1β and is associated with auto-inflammatory diseases, namely CAPS. Since IL-1β plays a central role in the pathogenesis of CASP, mutations and polymorphisms of NALP3 become one of the main subjects of studies. One of these polymorphisms is Q705K polymorphism in which glutamine is replaced by lysine. Verma et al.[14] demonstrated that this polymorphism is associated with high levels of IL-1 levels and chronic inflammation which can be blocked by anakinra.

Like auto-inflammatory diseases, it is documented that patients with BD also have high levels of IL-1β. Gene mutations and polymorphisms including IL-1β receive more attention in the pathogenesis of the disease.[15-17] Although Q705K polymorphism has been demonstrated in some chronic inflammatory diseases, to our knowledge, no study has been conducted focusing on BD. Therefore, in this study, we aimed to investigate Q705K polymorphism in NALP3 gene in BD.

PATIENTS AND METHODS

This case-control study was conducted at Marmara University Hospital between December 2008 and December 2009 and included 180 patients (92 males, 88 females; mean age 36.6±11.3 years; range 21 to 56 years) diagnosed as BD according to 1990 International Study Group criteria[18] and 168 control blood donors (92 males, 76 females; mean age 34.9±13.2 years; range 22 to 55 years). Local Ethics Committee approval was received and a written informed consent was obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

The characteristics, demographic, clinical, and laboratory parameters were recorded from the archives of rheumatology clinics. Cellular deoxyribonucleic acid (DNA) was isolated from peripheral blood using standard salting-out procedures. NALP3 Q705K gene polymorphism was determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism methods. Two oligonucleotide primers were used for DNA amplification. The forward primer was 5'AACTGTCATCGGGTGGAGTC 3' and the reverse primer was 5'CCTCATCCCATTTATCCACCT3'. Polymerase chain reaction amplification was performed in a total volume of 25 μL consisting of 0.2 mM deoxynucleotide, 2 mM magnesium chloride, 1x PCR (ammonium) buffer, 0.75 U Taq polymerase enzyme (MBI Fermentas-Germany) and 0.4 μM of each specific primer and 100 ng of genomic DNA were added to the final solution. The cyclic parameters consisted of first denaturation step of two minutes at 95 °C, total 30 cycles of steps and a final extension step of two minutes at 75 °C.

Restriction fragment length polymorphism analysis is based on digestion of PCR products by specific enzymes named as restriction enzymes. The restriction enzyme digests the PCR product at specific polymorphic region. In this study, the restriction enzyme appropriate for the studied polymorphism was identified by NEBcutter (New England BioLabs) program. The PCR products were incubated

TABLE 1

The characteristics of the patients

	Patients with Behçet's disease		Healthy controls	
	n	%	n	%
Gender				
Female	92	51	92	54.8
Male	88	49	76	45.2
Mean age (years)		36.6		34.9
Oral aphthous ulcers	173/173	100	-	-
Genital ulcers	135/173	78	-	-
Ocular lesions	64/176	36.4	-	-
Erythema nodosum	114/172	66.4	-	-
Gastrointestinal system involvement	3/170	1.8	-	-
Joint involvement	56/173	32.4	-	-
Neurologic involvement	13/173	7.5	-	-
Vascular involvement	55/176	31.2	-	-
Positive pathergy test	104/150	69.3	-	-
Family history	43/144	29.9	-	-
B51 positivity	117/177	66.1	25/95	26.3

at 37 °C for 16 hours with restriction enzyme for proper digestion. Digested products were run on 1.5% agarose gel stained with ethidium bromide. The gel was analyzed under ultraviolet light and three genotypes (AA, CC, CA) were determined where the variant allele was named as A.

Statistical analysis

The frequencies of allele and genotypes were determined by direct counting. The distribution of the allele and genotype frequencies was compared with K2-square, Fisher's exact, and chi-square tests between patients and controls. The risk ratios (odds ratio) and 95% confidence intervals were identified. The relationship between the clinical parameters and polymorphism was analyzed and *p* values less than 0.05 were accepted as statistically significant.

RESULTS

There was no statistically significant difference between groups in respect to mean ages (p=0.2). The characteristics of patients were documented in Table 1. Vascular involvement was statistically higher in male patients than in females (p=0.005).

Two (1.1%) homozygote polymorphic genotypes (AA genotype) were found among the 180 patients. Sixteen patients (8.9%) were heterozygote (CA) for the mentioned polymorphism. In control group, there were two homozygote polymorphic genotypes (1.2%) and the ratio of heterozygotes was 11.3%. There was no statistically significant difference between patients and controls in terms of genotypic distribution (p=0.75) (Figure 1). Subgroup analysis according the sex revealed no statistically significant difference (p=0.6).

We performed a subgroup analysis with regard to clinical presentation. Patients were grouped according to ocular, mucocutaneous, vascular, neurologic, and joint involvement, which revealed no statistical difference (Table 2).

The frequency of the polymorphic allele in patients was 0.055, while it was 0.068 in controls (p=0.6). Subgroup analysis including system/organ involvements revealed no differences between subgroups and controls (Table 3).

DISCUSSION

Although the pathogenesis of BD has not been well documented, genetic and environmental factors such as infectious agents are blamed.[3] Among the genetic factors, HLA-B51 was documented in many ethnic groups.[19] It was demonstrated that IL-1β has an important role in the onset and the clinical signs and symptoms of

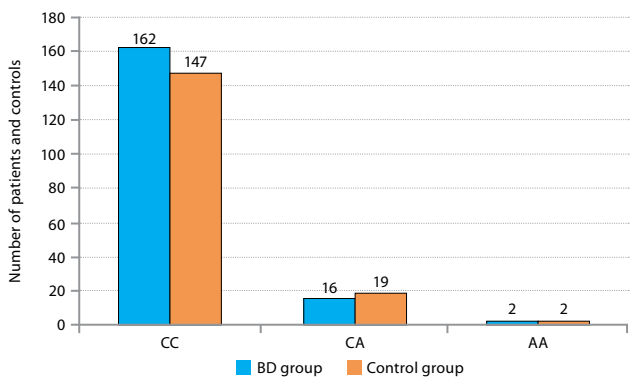

Figure 1. Polymorphism distribution in patients and healthy controls. BD: Behçet's disease; AA: Homozygote for polymorphism; CA: Heterozygote for polymorphism; CC: Wild type.

TABLE 2

Polymorphism distribution according to system involvement in Behçet's disease

System/organ involvement	Polymorphism	Number of patients	
		n	%
Ocular	CC	57	89.1
	CA	5	7.8
	AA	2	3.1
Joint	CC	44	80.4
	CA	9	16.1
	AA	2	3.6
Neurologic	CC	11	84.6
	CA	2	15.4
	AA	0	0
Vascular	CC	50	90.9
	CA	3	5.5
	AA	2	3.6
Mucocutaneous	CC	140	89.2
	CA	15	9.6
	AA	2	1.3

CC: Wild type; CA: Heterozygote for polymorphism; AA: Homozygote for polymorphism.

TABLE 3

The comparison of allele frequencies between subgroups and patients

Organ/system involvement	Allele frequency	*p*	χ^2
	Patients/Controls		
Ocular	0.07/0.073	0.94	0.005
Joint	0.13/0.073	0.11	2.58
Neurologic	0.08/0.073	0.69	-*
Vascular	0.06/0.073	0.86	0.03
Mucocuteneous	0.064/0.073	0.68	0.17

* Fischer's exact test was performed.

disease in BD and other chronic auto-inflammatory diseases, namely CAPS.[14,15] The inflammasome complex including NALP3, TUCAN (CARDS8) and ASC, activated caspase mediated IL-1β production and they became the focus of the studies.[13,14] Gain of function mutations and polymorphisms of these genes might lead to chronic inflammatory diseases. NALP3 Q705K gene polymorphism together with polymorphism in TUCAN gene C10X was demonstrated in a patient with arthritis and antibiotic resistant fever. The symptoms of the patient were related to high levels of IL-1 and gain of function of the inflammasome since all the symptoms regressed after treatment with anakinra, IL-1 receptor blocking agent.[20] Another disease associated with over production of IL-1β was Crohn's disease. Schoultz et al.[21] demonstrated that combined polymorphisms of NALP3 (Q705K) and (C10X) CARD8 had a role in development of Crohn's disease in Swedish males but not in females. The polymorphisms in CARD8 and NALP3 (Q705K) were also shown to be associated with rheumatoid arthritis.[22] In this study, the patients with both alleles had more severe disease and the rate of anti-tumor necrosis factor administration was higher in this group.[22]

It was also shown that IL-1 family has an important role in the pathogenesis of psoriasis. Day et al.[23] hypothesized that autoinflammatory genes including NLRP3 have a significant role in pathogenesis in psoriatic juvenile idiopathic arthritis that shared some common properties with auto-inflammatory diseases. Another example for diseases with chronic inflammation was gout and pseudogout caused by monosodium urate (MSU) or calcium pyrophosphate dihydrate crystals, respectively. Although the etiological agents were well documented, little was known about the mechanism. It was demonstrated that MSU crystals triggered the inflammatory response via caspases and NALP3.[24,25] NALP3 was also blamed to have a role in asbestosis and silicosis. Cassel et al.[26] showed that the inflammatory response and subsequent development of pulmonary fibrosis after inhalation of silica or asbestos were dependent on the NALP3 inflammasome.

In the literature, it was demonstrated that inflammasome and NALP3 have a role in many diseases accompanied by inflammation and IL-1 overproduction. Although BD has similar characteristics with auto-inflammatory diseases, to our knowledge, NALP3 Q705K gene polymorphism was not studied in BD so far. In our study, we found two patients and two healthy controls with homozygote genotype in regards to the Q705K polymorphism. There was no difference in rates of homozygotes and heterozygotes between patient and control groups (8.9% and 11.3%, respectively; p>0.05). Unlike in Crohn's disease, we could not find any difference when males and females were analyzed separately (p=0.6). Also, we could not document any significant difference in subgroup analysis according to system/organ involvements.

In our study, the allele frequencies in BD patients and controls were 5.5% and 6.5%, respectively. Similar to the results of our study, in Sweden, the allele frequency was 6.5%.[22] We could not determine a significant difference between BD patients and healthy controls in terms of allel frequency.

Our study has some limitations. The group was heterogenous with different system and organ invovement. The number of the patients with neurologic and gastrointestinal system involvement was relatively low.

In conclusion, NALP3 Q705K gene polymorphism did not increase the susceptibility of individuals to BD.

The polymorphism did not correlate with the disease severity, sex or organ involvement. The NALP3 Q705K gene polymorphism was mostly studied in conjunction with polymorphism in CARDS8. Thus, for clarification of the pathogenesis of BD, it might be useful to study polymorphisms affecting both genes.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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REFERENCES

1. Marshall SE. Behçet's disease. Best Pract Res Clin Rheumatol 2004;18:291-311.
2. Yurdakul S, Hamuryudan V, Yazici H. Behçet syndrome. Curr Opin Rheumatol 2004;16:38-42.
3. Direskeneli H. Behçet's disease: infectious aetiology, new autoantigens, and HLA-B51. Ann Rheum Dis 2001;60:996-1002.
4. Kaneko F, Oyama N, Nishibu A. Streptococcal infection in the pathogenesis of Behçet's disease and clinical effects of minocycline on the disease symptoms. Yonsei Med J 1997;38:444-54.
5. Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science 1999;285:727-9.
6. Verity DH, Wallace GR, Vaughan RW, Kondeatis E, Madanat W, Zureikat H, et al. HLA and tumour necrosis factor (TNF) polymorphisms in ocular Behçet's disease. Tissue Antigens 1999;54:264-72.
7. Takeuchi M, Kastner DL, Remmers EF. The immunogenetics of Behçet's disease: A comprehensive review. J Autoimmun 2015;64:137-48.
8. Gül A, Uyar FA, Inanç M, Ocal L, Barrett JH, Aral O, et al. A weak association of HLA-B*2702 with Behçet's disease. Genes Immun 2002;3:368-72.
9. Choukri F, Chakib A, Himmich H, Hüe S, Caillat-Zucman S. HLA-B*51 and B*15 alleles confer predisposition to Behçet's disease in Moroccan patients. Hum Immunol 2001;62:180-5.
10. Gül A. Behçet's disease as an autoinflammatory disorder. Curr Drug Targets Inflamm Allergy 2005;4:81-3.
11. Direskeneli H. Autoimmunity vs autoinflammation in Behcet's disease: do we oversimplify a complex disorder? Rheumatology (Oxford) 2006;45:1461-5.
12. Campbell L, Raheem I, Malemud CJ, Askari AD. The Relationship between NALP3 and Autoinflammatory Syndromes. Int J Mol Sci 2016;17.
13. Schroder K, Tschopp J. The inflammasomes. Cell 2010;140:821-32.
14. Verma D, Särndahl E, Andersson H, Eriksson P, Fredrikson M, Jönsson JI, et al. The Q705K polymorphism in NLRP3 is a gain-of-function alteration leading to excessive interleukin-1β and IL-18 production. PLoS One 2012;7:34977.
15. Yosipovitch G, Shohat B, Bshara J, Wysenbeek A, Weinberger A. Elevated serum interleukin 1 receptors and interleukin 1B in patients with Behçet's disease: correlations with disease activity and severity. Isr J Med Sci 1995;31:345-8.
16. Alayli G, Aydin F, Coban AY, Süllü Y, Cantürk F, Bek Y, et al. T helper 1 type cytokines polymorphisms: association with susceptibility to Behçet's disease. Clin Rheumatol 2007;26:1299-305.
17. 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.
18. Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. Lancet 1990;335:1078-80.
19. Lee YH, Song GG. Associations between major histocompatibility complex class I chain-related gene A polymorphisms and susceptibility to Behcet's disease. A meta-analysis. Z Rheumatol 2015;74:714-21.
20. Verma D, Lerm M, Blomgran Julinder R, Eriksson P, Söderkvist P, Särndahl E. Gene polymorphisms in the NALP3 inflammasome are associated with interleukin-1 production and severe inflammation: relation to common inflammatory diseases? Arthritis Rheum 2008;58:888-94.
21. Schoultz I, Verma D, Halfvarsson J, Törkvist L, Fredrikson M, Sjöqvist U, et al. Combined polymorphisms in genes encoding the inflammasome components NALP3 and CARD8 confer susceptibility to Crohn's disease in Swedish men. Am J Gastroenterol 2009;104:1180-8.
22. Kastbom A, Verma D, Eriksson P, Skogh T, Wingren G, Söderkvist P. Genetic variation in proteins of the cryopyrin inflammasome influences susceptibility and severity of rheumatoid arthritis (the Swedish TIRA project). Rheumatology (Oxford) 2008;47:415-7.
23. Groves RW, Mizutani H, Kieffer JD, Kupper TS. Inflammatory skin disease in transgenic mice that express high levels of interleukin 1 alpha in basal epidermis. Proc Natl Acad Sci U S A 1995;92:11874-8.
24. Martinon F, Pétrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 2006;440:237-41.
25. Pétrilli V, Martinon F. The inflammasome, autoinflammatory diseases, and gout. Joint Bone Spine 2007;74:571-6.
26. Cassel SL, Eisenbarth SC, Iyer SS, Sadler JJ, Colegio OR, Tephly LA, et al. The Nalp3 inflammasome is essential for the development of silicosis. Proc Natl Acad Sci U S A 2008;105:9035-40.