IL-6 receptor, IL-8 receptor and TNF-α238 (G/A) polymorphisms are not associated with Behçet's disease in patients of German or Turkish origin

K. Storz[1], J. Löffler[3], S. Koch[1], R. Vonthein[2], C.C. Zouboulis[4], I. Fresko[5], H. Yazici[5], I. Kötter[1]

[1]Department of Internal Medicine II and [2]Department of Medical Biometry, University Hospital, Tübingen; [3]Department of Haematology/Rheumatology, University Hospital, Würzburg; [3]Department of Medical Biometry, University Hospital, Tübingen; [4]Department of Dermatology and Department of Immunology, Dessau Medical Center, Dessau, Germany; [5]Department of Rheumatology, Cerraphasa University Hospital, Istanbul, Turkey.

Kristina Storz; Jürgen Löffler, PhD; Silvia Koch; Reinhard Vonthein; Christos C. Zouboulis, MD; Izzet Fresko, MD; Hasan Yazici, MD; Ina Kötter, MD.

Please address correspondence to: Dr. Ina Kötter, Associate Professor, Department of Internal Medicine II, Oncology, Haematology, Immunology, Rheumatology, Pulmology, Otfried-Müller-Str. 10, 72076 Tübingen, Germany. E-mail: ina.koetter@med.uni-tuebingen.de

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ABSTRACT

Objective. *To assess the association of polymorphisms of the IL-6 receptor gene (+24013A/G:Ala31Ala; +48892 A/C: Asp358Ala), the IL-8 receptor gene (+2607G/C:Ser/Thr IL-8RA), and TNF-α 238 (G/A) single nucleotide polymorphism (SNP) with Behçet's disease in patients of German or Turkish origin.*

Methods. *DNA was extracted from blood samples taken from patients in Germany (n=93) and Turkey (n=28), as well as from 51 German and 20 Turkish healthy controls. The polymorphisms were analysed by PCR with the LightCycler system.*

Results. *No significant association was found between TNF-α 238, +2607 IL-8RA, +48892 IL-6R or +24013 IL-6R-polymorphisms and nationality or disease. Statistically there was no difference between the patients and controls (TNF-α 238: p = 0.86; +2607 IL-8RA: p=0.23; +48892 IL-6R: p=0.087; +24013 IL-6R: p = 0.80) nor between Germans and Turks (TNF-α 238: p=0.13; +2607 IL-8RA: p=0.68; +48892 IL-6R: p=0.32; +24013 IL-6R: p=0.65).*

Conclusion. *The single nucleotide polymorphisms of the IL-6 and IL-8 receptor genes and the TNF-α gene analysed here do not appear to be associated with Behçet's disease.*

Introduction

Behçet's disease (BD) is a chronic inflammatory disorder characterised mainly by recurrent oral and genital ulcerations, various skin lesions, and uveitis (1). Its exact etiopathogenesis remains unknown, although an association of HLA-B51 with BD has been described in many ethnic groups and is the strongest genetic finding so far (1, 2). Several immunological particularities have been observed in patients with active BD, including elevated serum levels of proinflammatory cytokines such as TNF-α, IL-6 and IL-8 (1, 3). Furthermore, IL-6 and IL-8 gene polymorphisms are known to play a role in chronic inflammatory and autoimmune diseases (1, 4).

IL-6 is a pleiotropic cytokine and an important mediator of inflammatory and immune responses. It is encoded on chromosome 7p21 and interacts with the IL-6 receptor, which consists of an alpha-chain (IL6RA) and a subunit (gp130), and is located on chromosome 1q21 (5).

IL-8 (also called CXCL8), is a member of the CXC chemokine family and is a potent neutrophil and lymphocyte chemoattractant. Its cellular activities are mediated by two receptors, IL8RA (CXCR1) and IL8RB (CXCR2), which are encoded on chromosome 2q34-35 (4). IL-8 levels are elevated in the serum of BD patients and neutrophil hyperfunction is one pathogenic feature of the disease (6-8).

TNF-α is encoded in the class III region of the HLA complex adjacent to HLA-B on chromosome 6p21, and 12 single nucleotide polymorphisms (SNP) have been described so far, located on the promoter-, exon-, intron- and 3'-regions (2).

TNF-α is elevated in the serum as well as intra-cellularly in BD (2) and TNF-α antagonists are effective in the treatment of BD (9). The polymorphism examined here [TNF-α 238 (G/A)] was recently described to be associated with increased TNF-α gene transcription and serum levels, and an association with psoriasis has been reported (10).

In addition, we analysed a polymorphism of the IL-8 receptor gene IL-8RA (+2607B/C:Ser/Thr), which was selected because IL-8RA exclusively binds

IL-8 whereas IL-8RB also binds other related CXC chemokines, such as the growth-related oncogene (GRO) family of proteins (11). Also studied were two IL-6 receptor gene IL-6R polymorphisms (+24013A/G:Ala31Ala; +48892 A/C:Asp358Ala), because the 48892A/C polymorphism is known to functionally increase serum IL-6 and soluble IL-6R levels (12, 13). Neither these IL-6R polymorphisms nor the IL-8RA gene polymorphism have yet been examined in BD.

Materials and methods

After obtaining approval from the local ethics committee and informed written consent from the study participants, blood samples were taken from 93 German and 28 Turkish patients diagnosed with BD according to the International Study Group Classification Criteria (14). Blood samples were also obtained from healthy controls (51 German and 20 Turkish subjects) whose distribution in terms of age and sex was similar to that of the patient cohort. Self-declared nationality was used as a proxy for ethnicity, as it coincides in more than 95% of all cases.

Genomic DNA was purified using the QIAGEN DNA Blood Mini Kit® (Qiagen, Hilden, Germany). Genotyping of the TNF-α 238, IL-8RA (+2607B/C:Ser/Thr) and IL-6R (+24013A/G:Ala31Ala; +48892 A/C:Asp358Ala) SNPs was performed by melting curve analysis using Light Cycler® technology (Roche Diagnostics, Mannheim, Germany). The primer sequences for TNF-α 238 were 5' – CCTgCATCCTgTCTggAAgTTA and 5' – CCTgCATCCTgTCTggAAgTTA, for +2607 IL-8RA they were 5' – gCCAAgAACTCCTTgCTgAC and 5' – CTggCTgCCCTACAACC, for +48892 IL-6R they were 5' – CATCTCACCTCAgAACAATgg and 5' – TCCTTTgAggCTTTTgACA, and for +24013 IL-6R they were 5' – CCAgTgAACAgTggCATT and 5' – TgggCCACTTCATCATTATCA. A single PCR protocol and thermocycling program was used.

Allele and genotype frequencies in the patient and control groups were counted and contingency tables were prepared, which were displayed schematically as mosaic plots. Allele and genotype frequencies between the healthy controls and the BD patients were tested for equality using the likelihood ratio χ^2-test. The unadjusted p-values are given. In the case of multiple small p-values, they were compared to local significance levels, adjusted using the Bonferroni-Holm procedure. Odds ratios (OR) and 95% confidence intervals were calculated for each polymorphism. All statistical analyses were performed using JMP (Version 5.1).

Results

The percentages of patients versus controls by nationality (German or Turkish) for the TNF-α 238 polymorphism were as follows: 85% of the German patients were homozygous for the wild type (WT) *vs*. 86% of the German controls; 15% of the German patients *vs*. 16% of the respective controls were heterozygous; Turkish patients were homozygous for the WT in 96% *vs*. 90% of the Turkish controls; and 4% *vs*. 10% were heterozygous. None of the probes were homozygous for the polymorphism.

For the IL-8 polymorphism, 90% of German patients were homozygous for the WT *vs*. 85% of the German controls; 10% of German patients were heterozygous compared to 15% of the respective controls. 90% of Turkish patients were homozygous for the WT *vs*. 80% of the controls; 10% of Turkish patients and 20% of Turkish controls were heterozygous.

For the +48892 IL-6R polymorphism, 28% of the subjects from each control group (German and Turkish) were homozygous for the polymorphism. Similarly, 25% of the Turkish patients and 38% of the German patients were homozygous for the polymorphism. 56% of the German and Turkish controls were heterozygous, while 16% were homozygous for the WT. 38% *vs*. 25% of the German and Turkish patients were homozygous for the polymorphism. For the +24013 IL-6R polymorphism, no heterozygous genotype could be found. A few patients and controls were homozygous for the polymorphism (2% of the German and none of the Turkish patients, 4% of German and 3% of the Turkish controls). All other samples were homozygous for the WT (Tables I-IV).

For the statistical analysis, allele frequencies between Germans and Turks (patients and controls evaluated together) and patients and controls (Germans and Turks evaluated together) were used for each polymorphism. None of the polymorphisms revealed a significant association with nationality or disease:

TNF-α 238: Germans versus Turks (p=0.13; OR=0.39, [0.089 to 1.15]); patients versus controls p=0.86, OR=1.08, [0.44 to 2.5] +2607.

IL-8RA: Germans versus Turks p = 0.68, OR = 1.2, [0.45 to 3.0]; patients versus controls p=0.24, OR=1.7, [0.704 to 3.9] +48892.

IL-6R: Germans versus Turks p=0.32, OR=1.27 [0.80 to 2.06]; patients versus controls p=0.087, OR=0.69, [0.45 to 1.06]. +24013 IL-6R: Germans versus Turks p=0.65, OR=0.70, [0.104 to 2.8]; and patients versus controls p=0.81, OR=1.18, [0.30 to 4.2].

Since no test was significant at the local 5% level, no set of tests was significant on the multiple 5% level.

Discussion

In the pathogenesis of BD, genetic, environmental and immunological factors have been discussed (1). The contribution of genetic factors is estimated to be approximately 30% (3), and multiple host genetic factors have been proposed.

In order to identify new susceptibility genes, we investigated TNF-α 238, +2607 IL-8RA, +48892 IL-6R and +24013 IL-6R gene polymorphisms on the assumption that polymorphisms in either the cytokine or cytokine-receptor genes, or both, may influence the expression and activity of the corresponding cytokines. The critical role of TNF-α in patients with BD is well documented (2), although the functional role of TNF-α 238 promoter polymorphism remains unsolved; some studies report increased transcriptional activity and the release of TNF-α, whereas others have been unable to confirm these results (15, 16). Furthermore a repressor site between nucleotides -254 and

-230, exactly where the TNF-α 238 polymorphism maps, has been identified (17). This could push genetic transcription towards increased gene regulation, on the one hand due to a switch of guanine to adenine, and on the other hand due to a better interaction of the guanine allele with a positive regulator of TNF-α production.

However, in this study we were unable to show any association between TNF-α 238 gene polymorphism and BD (p=0.8649). This result is in contrast to the only other study focusing on the possible association of TNF-α 238 polymorphism with BD. Ahmad *et al*. reported a link between this polymorphism and BD (p=0.008) (18). However, they studied 133 white Caucasoid patients in the UK (whose exact ethnic origin is not stated in the paper) and 354 healthy controls. The different ethnicity of the patients could be one reason for the conflicting results between our study and theirs. Furthermore, they conducted haplotype studies that concentrated mainly on HLA gene associations; hence, for methodological reasons their results may not be comparable to ours, which were obtained by PCR.

Ahmad *et al*. also found a strong association with -1031 TNF polymorphism, a result that was confirmed by a recently published paper in which Akman *et al*. reported a similar association in Turkish patients with BD (p=0.018) (18, 19). The problem is that TNF-α polymorphisms are located in a highly polymorphic region and are in strong linkage disequilibrium with the HLA genes and with each other (20). Hence it is generally difficult to prove individual genetic polymorphisms in this region.

Elevated IL-8 serum levels in patients with BD were described to be correlated with disease activity. This could be genetically determined due to the differential expression of the chemokine, the cytokine, or its receptors or both (21). To the best of our knowledge, only one study – which investigated the possible role of IL-8 and IL-8 receptor (IL-8R) polymorphisms in the pathogenesis of BD – has been published. However, Dymaz-Tozkir *et al*. did not find any association between IL-8 gene and IL-8RB gene polymorphisms and BD, even though they studied 3 SNPs of the IL-8 gene (-353 A/G, +1530 T/C, +3331 A/G) and 2 SNPs of the IL-8RB gene (+785 C/T, +1208 T/C) (22).

In the present study, we examined the IL-8 receptor gene polymorphism +2607 IL-8RA (G/A α → Ser/Thr), hypothesizing that this as yet unexamined polymorphism could be a factor in the development of BD. However, we failed to find any association (p=0.2378). Another study by Lee *et al*. explored the haplotype association of the IL-8 gene with BD, and reported no links between BD and CXCR-1 +2607 G/C polymorphism, nor with any of the other single SNPs of the IL-8 gene or the CXCR-2 gene (23). However, this study did show that one haplotype was significantly more frequent in patients than in controls. The CXCR-1 +2607 G/C polymorphism was also examined in a study on systemic sclerosis, and no association with fibrosing alveolitis could be demonstrated, whereas a significant increase in the frequency of two CXCR-2 SNPs was found (24).

We examined both IL-6R SNP [+48892 IL6R (A/C → Asp/Ala) and +24013 (G/A → Ala/Ala)], in the belief that polymorphisms in the receptor gene could play a role in the development of BD, especially in the light of a study showing an association of the IL-6R 48892 A/C polymorphism with parodontitis (25). Chang *et al*. analysed specific SNPs in the IL-6 gene, but could not

Table I. Genotype distribution of TNF-α 238 in German and Turkish patients and controls (WT = wildtype).

TNF-α 238 Genotype	Patients (%)		Controls (%)	
	92 Germans	30 Turks	51 Germans	20 Turks
AA	0 (0)	0 (0)	0 (0)	0 (0)
GA	14 (15)	1 (4)	7 (15)	2 (10)
GG (WT)	78 (85)	29 (96)	44 (85)	18 (90)

Table II. Genotype distribution of +2607 IL-8RA in German and Turkish patients and controls (WT = wildtype)

+2607 IL-8RA Genotype	Patients (%)		Controls (%)	
	94 Germans	30 Turks	50 Germans	20 Turks
CC	0 (0)	0 (0)	0 (0)	0 (0)
GC	9 (10)	3 (10)	7 (15)	4 (20)
GG (WT)	85 (90)	27 (90)	43 (85)	16 (80)

Table III. Genotype distribution of +48892 IL-6R in German and Turkish patients and controls (WT = wildtype).

+48892 IL-6R Genotype	Patients (%)		Controls (%)	
	94 Germans	30 Turks	50 Germans	20 Turks
CC	36 (38)	14 (25)	14 (25)	5 (28)
AC	47 (50)	14 (70)	28 (70)	14 (56)
AA (WT)	11 (12)	2 (5)	8 (5)	11 (16)

Table IV. Genotype distribution of +24013 IL-6R in German and Turkish patients and controls (WT = wildtype).

+24013 IL-6R Genotype	Patients (%)		Controls (%)	
	94 Germans	30 Turks	50 Germans	20 Turks
AA	2 (2)	1 (3)	2 (4)	0 (0)
GA	0 (0)	0 (0)	0 (0)	0 (0)
GG (WT)	2 (98)	29 (97)	48 (96)	20 (100)

find an association with BD (26). In the present study no association between BD and the IL-6 receptor gene polymorphisms +48892 IL-6RA and +24013 IL-6RA was found (p=0.3164 and p=0.7989). To the best of our knowledge, no other studies focusing on IL-6R polymorphisms and BD have been published to date.

In conclusion, none of the polymorphisms analysed here appear to be associated with BD in German or Turkish patients. However, the patient samples were relatively small, which could have influenced the results by limiting their statistical power. This is a problem with almost all of the studies on SNPs and BD published thus far. According to a recent paper by Kraft *et al*., the power calculation for a rare disease with a low population prevalence should be different from that for a common disorder with a high population prevalence (27). However, the prevalence of BD varies substantially in different populations (0.6/100,000 in Germany *vs*. up to 360/100,000 in Turkey), which makes the calculation of the required sample size difficult. Hence, to achieve reliable results, international co-operation is necessary in order that very large patient samples with ethnically matched healthy controls can be collected and compared, including the frequencies we report here.

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