Interleukin-18 gene polymorphisms in Korean patients with Behçet's disease

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ABSTRACT

Objective. *There is strong evidence that Th1-type cytokines play an important role in the pathogenesis of Behçet's disease (BD). Interleukin (IL)-18 is a proinflammatory cytokine that mediates Th1-polarized immune responses, and elevated levels of IL-18 have been observed in the sera and bronchoalveolar lavage fluid of patients with active BD. Therefore, the aim of this study was to investigate the potential associations of two single nucleotide polymorphisms (SNPs) at positions –137 (G/C) and –607 (C/A) in the promoter region of the IL-18 gene with a susceptibility to BD in the Korean population.*

Methods. *Ninety-eight patients with BD and 105 healthy controls were studied. All of the subjects were genotyped using sequence specific PCR. The genotypes and alleles between patients with BD and controls were compared using the χ^2 test, together with Yate's correction where appropriate. Haplotype analysis was assessed using the EH program.*

Results. *The genotype and allele distributions of the two SNPs did not differ significantly between patients with BD and controls. The haplotype frequencies of the IL-18 promoter polymorphisms were also similar between patients with BD and controls. However, the frequency of the GG genotype at position –137 was significantly higher in BD patients with ocular lesions than in those without ocular lesions ($p = 0.026$, $p_c = 0.048$, $OR = 4.1$).*

Conclusion. *Although the IL-18 gene polymorphisms were not associated with a susceptibility to BD in the Korean population, the patients carrying the GG genotype at position –137 had a higher risk of developing the ocular lesions. Further studies in other populations are required to confirm these results.*

Introduction

Behçet's disease (BD) is a chronic inflammatory disorder, involving several organs. Although the exact pathogenesis for BD is not completely understood, it has been suggested that the disease is triggered in genetically susceptible individuals by environmental factors, such as infectious agents (1). Although the HLA-B51 is known to be the candidate gene showing the strongest association with BD (1-4), the contribution of the HLA-B locus to the overall genetic susceptibility to BD has been estimated as being less than 20% in a multi-case familial study (5). This finding suggests that genetic factors other than HLA-B51 are involved in the pathogenesis of BD. In addition, evidence has emerged that polymorphisms in the cytokine genes are involved in modifying immune responses. Recently, we have reported that IL-6 gene polymorphism is an additional susceptibility factor to BD in the Korean population (6).

Interleukin (IL)-18, which is a member of the IL-1 family, is a pleotropic and proinflammatory cytokine that is produced mainly by activated macrophages, and which participates in both innate and acquired immune responses. IL-18 plays a pivotal role in the T helper 1 (Th1)-type response, principally owing to its ability to induce interferon-γ (IFN-γ) production in T cells and natural killer (NK) cells (7, 8). The promotion of Th1 and NK cell responses is mediated primarily in synergy with IL-12 (9). It has been noted that IL-18 is expressed at chronic inflammatory sites of Th1-type autoimmune diseases in humans, such as rheumatoid arthritis (10), Crohn's disease (11), and multiple sclerosis (12).

Three single nucleotide polymorphisms (SNPs) in the promoter and two SNPs in the 5'-nontranslated regions of

the IL-18 gene, which is located on chromosome 11q22.2-q22.3, have recently been identified. Of these polymorphisms, the two SNPs at positions –137 (G/C) and –607 (C/A) in the promoter have been reported to affect transcription factor binding and gene activity (13) and to be associated with various chronic inflammatory and infectious diseases, such as type I diabetes (14, 15), sarcoidosis (16), and necrotizing enterocolitis (17).

Elevated levels of IL-18 have been observed in the sera and bronchoalveolar lavage (BAL) fluid of patients with active BD (18, 19). Moreover, it has been noted that recombinant IL-18 induces IFN-γ production from the BAL fluid cells isolated from patients with BD (18). In addition, it is well known that abnormalities of both innate and acquired immune responses are involved in the pathogenesis of BD, and that Th1 type cytokines play an important role in the development of inflammation in BD (1). These findings have led to the hypothesis that the altered IL-18 activity caused by IL-18 gene polymorphisms may play a specific role in a susceptibility to BD. Therefore, the aim of this study was to investigate the potential associations of the two SNPs at positions –137 and –607 in the promoter region of the IL-18 gene with a susceptibility to or clinical manifestations of, BD in the Korean population.

Patients and methods

Subjects

The study population included 98 patients with BD (41 males and 57 females) who fulfilled the International Study Group (ISG) criteria (20), and 105 healthy controls (40 males and 65 females). All of the subjects were ethnically homogenous Korean, and unrelated to each other. The mean ages of the BD group and controls were 38.5 years (± 9.0) and 40.0 years (± 5.5), respectively. The presence of one or more of the following clinical features during the course of the disease was regarded as a severe manifestation (21, 22): posterior uveitis or retinal vasculitis, gastrointestinal ulcerations with bleeding or perforation, major vessel involvement, and major organ involvement, such as central nervous system, heart, or kidney. The mean age at onset, which was defined as the time when the patient fulfilled the ISG criteria, was 32.7 years (±8.8) in the BD group. The mean disease duration in the BD group was 5.8 years (± 5.7). Informed consent was obtained from each subject.

DNA extraction

Genomic DNA was isolated from peripheral blood leukocytes using the Chelex extraction method (23).

Sequence specific PCR

The genotyping was performed using sequence specific PCR, as described previously (13). Briefly, for position -607, the common reverse primer (5'-TAACCTCATTCAGGACTTCC-3') and two sequence-specific forward primers (5'-GTTGCAGAAAGTGTAAAAATTATTAC-3' and 5'-GTTGCAGAAAGTGTAAAAATTATTAA-3') were used to amplify the 196-bp product. The control forward primer (5'-CTTTGCTATCATTCCAGGAA-3') was used to amplify the 301-bp fragment covering the polymorphic site as an internal positive amplification control. For position -137, the common reverse primer (5'-AGGAGGGCAAAATGC ACTGG-3') and two sequence-specific forward primers (5'-CCCCAACTTTTACGGAAGAAAAG-3' and 5'-CCCCAACTTTTACGGAAGAAAAC-3') were used to amplify the 261-bp product. The control forward primer (5'-CCAATAGGACTGATTATTCCGCA-3') was used to amplify the 446-bp fragment covering the polymorphic site as an internal positive amplification control. All of the PCR products were separated in 2% agarose gels that were strained with ethidium bromide.

Analysis of HLA-B51 antigen

HLA-B51 typing was performed in the BD group and controls by a two-step PCR with sequence-specific primers, as described in our previous study (4).

Statistical analysis

The data were analyzed using the SPSS statistical package program version 11.5 for Windows (SPSS Inc., Chicago, IL, USA). The genotype, allele, and haplotype frequencies between patients with BD and controls were compared by the two-tailed χ^2 test and, when indicated, the Yate's correction was performed (p_c). The same tests were used to examine the potential associations between the genotypes and the parameters of sex, clinical features, severe manifestations, or HLA-B51 positivity. The results obtained were re-evaluated by multiple logistic regression analysis when necessary. Comparisons with respect to disease duration and mean age at onset were performed by the t-test. Statistical significance was defined as $p \leq 0.05$. Haplotype and linkage disequilibrium (LD) analyses were assessed using the EH program (24-26). The risk for the disease susceptibility owing to the presence of the individual genotype or allele was calculated as the odds ratio (OR), which is presented along with the 95% confidence interval (CI) when indicated.

Results

Table I presents the clinical features of patients with BD. The genotype and allele frequencies of the two SNPs at positions –137 (G/C) and –607 (C/A) in the promoter region of the IL-18 gene are shown in Table II. The distributions of genotypes and alleles of these two polymorphisms did not differ significantly between patients with BD and control subjects (all $p > 0.05$). As for the SNP at position –137, although the frequencies of the GG genotype and G allele were somewhat higher in patients with BD than in controls, they did not reach statistical significance (p

Table I. Clinical features of 98 patients with Behçet's disease.

Clinical features	No. of patients (%)
Oral ulcerations	98 (100)
Skin lesions	91 (92.9)
Genital ulcerations	78 (79.6)
Positive pathergy test	35 (35.7)
Ocular lesions	29 (29.6)
Intestinal lesions	18 (18.4)
Peripheral arthritis	20 (20.4)
Vascular lesions	14 (14.3)
Central nervous system lesions	7 (7.1)
Positive HLA-B51	52 (53.1)

Table II. The genotype and allele frequencies of IL-18 promoter polymorphisms in the Behçet's group and control subjects.

	Controls No (%)	Behçet's group No (%)
Position –137		
Genotype		
GG	68 (64.8)	73 (74.5)
GC	37 (35.2)	25 (25.5)*
Allele		
G	173 (82.4)	171 (87.2)
C	37 (17.6)	25 (12.8)**
Position –607		
Genotype		
CC	14 (13.3)	22 (22.4)
AC	91 (86.7)	76 (77.6)†
Allele		
C	119 (56.7)	120 (61.2)
A	91 (43.3)	76 (38.6)‡

*p = 0.133 and **p = 0.173 for comparison of polymorphism at position -137 between Behçet's group and controls; p = 0.089 and p = 0.351 for comparisons of polymorphism at position -607 between Behçet's group and controls.

Table III. Association between study groups and the genotype frequencies at position –607 after stratification of the subjects based on the results of HLA-B51 testing.

	HLA-B51 negativity		HLA-B51 positivity	
	Control No. (%)	BD No. (%)	Control No. (%)	BD No. (%)
Genotype				
CC	12 (13.2)	12 (26.1)	2 (14.3)	10 (19.2)
AC	79 (86.8)	34 (73.9)*	12 (85.7)	42 (80.8)**

*p: 0.061 (p_c = 0.101) between Behçet's group and controls in the HLA-B51-negative subjects; **p: 0.670 for comparison between Behçet's group and controls in the HLA-B51-positive subjects.

> 0.05). In the cases of the SNPat position –607, although the frequencies of the CC genotype and C allele were higher in patients with BD than in controls, the results were not statistically different (p>0.05). Interestingly, the mutant homozygous genotypes, -137 CC and –607 AA, were absent from the Korean populations in our study.

We next studied the relationships between the clinical variables of BD and the two SNPs in the IL-18 gene promoter. No significant associations were found between the genotypes of the two SNPs and the clinical variables of the BD patients, *i.e.*, disease duration, mean age at onset, and the other clinical variables presented in Table I (with the exception of ocular lesions), as well as HLA-B51 positivity (p > 0.05 in all cases; data not shown). However, the distribution of genotypes at position –137 differed significantly between BD patients with ocular lesions and those without ocular lesions. In particular, the frequency of the GG genotype was much higher in BD patients with ocular lesions than in patients without ocular lesions (GG: 89.7% vs. 68.1%; GC: 10.3% vs. 31.9%, respectively; p = 0.026, p_c = 0.048). The OR value for the development of the ocular lesions in BD patients carrying the GG genotype was 4.1 (95% CI, 1.1 to 14.9). In addition, when multiple logistic regression analysis using the Enter method was performed to evaluate the effect of the patients' characteristics, including age, sex, the disease duration, and clinical variables presented in Table I, the association between the distribution of the genotypes at position –137 and the ocular lesions in BD remained statistically significant (β coefficient = 2.323, p= 0.028) (data not shown). On the other hand, severe manifestations were observed for 36 patients with BD, and there were no significant differences between BD patients with severe manifestations and those without severe manifestations in terms of the distribution of the genotypes of the two SNPs in the IL-18 gene promoter (data not shown).

When the studied subjects were stratified according to the results of HLA-B51 testing, the distribution of genotypes at position –137 did not differ significantly between patients with BD and controls, in either the HLA-B51-positive or HLA-B51-negative subgroups (data not shown). In the case of polymorphism at position –607, although patients with BD tended to exhibit a higher frequency of the CC genotype as compared to control subjects in the HLA-B51-negative subgroup, this difference did not reach statistical significance (p = 0.061; p_c = 0.101; OR = 2.3; 95% CI, 0.9 to 5.7) (Table III). On the other hand, when the haplotype frequencies of the IL-18 gene promoter were analyzed using the EH program, no significant differences were found between patients with BD and control subjects. Although the haplotype frequency consisting of –137 G and –607 C was somewhat higher in Behçet's group compared to controls, a significant difference was not observed (p > 0.05) (Table IV). In addition, the EH program revealed a D value of 0.9, which suggests the presence of a strong LD between the two SNPs at positions –137 and –607.

Discussion

BD is a chronic inflammatory disorder that is characterized by recurrent oral and genital ulcerations, as well as ocular and skin lesions. Although the precise nature of pathogenesis remains unclear, it is believed that diverse genetic and environmental factors contribute to the development of an inflammatory response in BD (1). In particular, the finding of a Turkish study that the sibling recurrence risk ratio (λs) was 11.4 – 52.5 (27) strongly implicates a genetic factor in the pathogenesis of BD. In

Table IV. Haplotype analysis between the two single nucleotide polymorphisms of the IL-18 gene promoter.

	control subjects	Behçet's group	Pvalue
–137 G/ –607 C	0.55753	0.61223	0.264
–137 G/ –607 A	0.26628	0.26022	0.891
–137 C/ –607 C	0.00913	0.00001	0.183
–137 C/ –607 A	0.16706	0.12754	0.262

addition to HLA-B51, which is considered the most relevant genetic factor, other major histocompatibility complex (MHC) genes, which include TNF gene polymorphisms (28) and the MHC class I chain-related gene A (1, 2), and non-MHC genes have been described as contributing to the pathogenesis of the disease. Recently, we reported that IL-6 gene polymorphisms (6) and endothelial nitric oxide synthase gene polymorphisms (22), both of which are non-MHC genes, are implicated in a susceptibility to BD in the Korean population. In the current study, the two SNPs at positions –137 (G/C) and –607 (C/A) in the promoter of the IL-18 gene, which is a non-MHC gene, were not associated with a susceptibility to BD in the Korean population. However, BD patients carrying the GG genotype at position –137 had a higher risk of developing the ocular lesions (OR = 4.1). As far as we are aware, this is the first investigation that addresses an association between IL-18 gene polymorphisms and a susceptibility to BD.

There is strong evidence that Th1 cytokine-producing cells play an important role in the pathogenesis of inflammation in BD. The frequency of Th1 type cytokines (IL-2 and IFN-γ)-producing T cells is increased in patients with active BD, as seen in flow cytometry studies for the intracytoplasmic cytokine expression levels in individual cell (1, 29, 30). Furthermore, the serum levels of IL-12, which is a critical cytokine for differentiation into a Th-1 type cell, is also elevated in parallel with increases of peripheral Th-1 lymphocytes and disease progression in BD (29). On the other hand, IL-18 is a proinflammatory cytokine that plays a key role in the Th1 response, primarily by inducing IFN-γ production in T cells and NK cells by virtue of synergy with IL-12 (7, 8). This synergism is based on the ability of IL-12 to upregulate IL-18 receptor expression on target cells (9). We therefore considered that the IL-18 gene polymorphisms might be an attractive candidate gene for BD.

Recently, it has been demonstrated that IL-18 gene expression is regulated by the IL-18 gene promoter and that two SNPs at positions –137 and –607 in this gene promoter affect the H4TF-1 nuclear factor binding site and cAMP-responsive element-binding protein binding site, respectively (13, 31). In addition, IL-18 promoter transcription assay showed high promoter activity for the G allele at position –137 and the C allele at position –607 (13). The subjects with these alleles associated with high promoter activity may have an increased expression of IL-18, inducing up-regulation of the IFN-r producing T-cells. Therefore, it seems plausible that patients with BD would have higher frequencies of the G allele at position –137 and/or the C allele at position –607.

In the current study, although the results did not reach statistical significance, patients with BD had somewhat higher frequencies of the G allele at position –137 and the C allele at position –607, as well as the haplotype consisting of these two alleles. Likewise, although the frequency of the CC genotype at position -607 was marginally increased for patients with BD than for controls in the HLA-B51-negative subgroup, statistically significant difference was not observed (p = 0.061; pc = 0.101; OR = 2.3). More investigations in a greater number of subjects are required to confirm these results. On the other hand, considerable inter-ethnic variability has been reported for the distributions of genotypes of the IL-18 promoter polymorphisms (13-16, 32). As in our population, the mutant homozygous genotypes, -137 CC and –607 AA, have not been observed in other ethnic groups (32).

In the present study, although we failed to detect significant associations between the IL-18 promoter polymorphisms and a susceptibility to BD, our findings suggest that the GG genotype at position –137 may be a risk factor for the ocular lesions associated with BD. This is consistent with the previous finding showing that subjects homozygous for G at position –137 had higher levels of IL-18 mRNA when compared with other genotypes (13). Therefore, we speculate that overexpression of IL-18 associated with the GG genotype at position –137 may cause IFN-γ production in T cells and NK cells in local cellular environment like eye, resulting in the development of the ocular lesions in BD. In addition, our study shows a strong LD between the two SNPs at positions –137 and –607, which raises some uncertainty as to whether the association between IL-18 polymorphism and the ocular lesions in BD is owing to primary association or to a nearby causative polymorphism.

In summary, although there was no evidence for a genetic association conferred by the two SNPs at positions –137 and –607 in the promoter region of the IL-18 gene with respect to a susceptibility to BD, BD patients carrying the GG genotype at position –137 had a higher risk of developing the ocular lesions. Further investigations in other ethnic groups and larger studies are required to provide more conclusive evidence regarding the role of the IL-18 gene polymorphisms in BD.

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