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Serum IL-33 level and IL-33 gene polymorphisms in Behçet's disease

Suleyman Serdar Koca · Murat Kara · Firat Deniz · Metin Ozgen · Caner Feyzi Demir · Nevin Ilhan · Ahmet Isik

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Abstract Behçet's disease (BD) is a chronic inflammatory disease. Increased productions of cytokines including interleukin (IL)-1β and IL-18 are documented, and IL-1α and β gene polymorphisms are associated with susceptibility to the disease. IL-33 is a recently discovered member of IL-1 cytokine family. The aim of the study was to detect serum IL-33 level and IL-33 gene polymorphisms in a cohort of BD. Unrelated 117 patients with BD and 149 healthy controls (HC) were enrolled. Serum IL-33 levels were analyzed by enzyme-linked immunosorbent assay method. DNA samples were harvested using an appropriate commercial DNA isolation kit. Four single nucleotide polymorphisms of IL-33 gene (rs7044343, rs1157505, rs11792633 and rs1929992) were genotyped using the appropriate commercial primer/probe sets on *real-time* PCR. Serum IL-33 level was not significantly different in the BD and HC groups ($p > 0.05$). However, its level was lower in the active BD patients compared to the inactive ones and HC group ($p = 0.044$ and $p = 0.037$, respectively). There was no significant difference in terms of the genotypic and allelic distributions of rs1157505 and rs1929992 polymorphisms ($p > 0.05$ for all). However, the TT variants of rs7044343 and rs11792633 polymorphisms were very rare, and the T allele frequencies of these polymorphisms were lower, in the BD group compared to the HC group ($p < 0.0001$ for all). The rs7044343 and rs11792633 variants of IL-33 gene are associated with the decreased risk of BD in our cohort. Therefore, it may be concluded that IL-33 acts a protective role on the pathogenesis of BD.

Keywords Behçet's disease · IL-33 · Polymorphisms

S. S. Koca (✉) · A. Isik
Department of Rheumatology, Faculty of Medicine, Firat University, Tip Fakultesi, Romatoloji BD, 23119 Elazig, Turkey
e-mail: kocassk@yahoo.com

M. Kara
Department of Medical Genetics, Faculty of Medicine, Mugla Sitki Kocman University, Mugla, Turkey

F. Deniz
Department of Internal Medicine, Faculty of Medicine, Firat University, Elazig, Turkey

M. Ozgen
Department of Rheumatology, Faculty of Medicine, 19 Mayis University, Malatya, Turkey

C. F. Demir
Department of Neurology, Faculty of Medicine, Firat University, Elazig, Turkey

N. Ilhan
Department of Biochemistry, Faculty of Medicine, Firat University, Elazig, Turkey

Introduction

Behçet' disease (BD) a multisystemic vasculitis is characterized by mucocutaneous, ocular, arthritic, and vascular manifestations. It has high prevalence in all along the ancient *Silk Road* [1, 2]. Although the etiopathogenesis of BD remains uncertain, immunological abnormalities including innate and adaptive immunity in humoral and cellular immunity have been supposed to be the cornerstone of the pathogenesis of BD [2, 3]. A variety of cytokines such as IL-6, IL-17, IL-18 and IL-21 are increased in BD; moreover, the numerous polymorphisms of cytokine gene including TNF-α, IL-1, IL-12, IL-23 and IFNγ are also associated with the disease [2, 3].

IL-33 [IL-1F11 or *nuclear factor from high endothelial venules* (NF-HEV)] is recently identified a cytokine from

IL-1 family [4]. Its level has been reported to be increased in several inflammatory diseases including rheumatoid arthritis (RA) [5–7], systemic sclerosis [8] and systemic lupus erythematosus (SLE) [9]. IL-33 gene is localized at 9p24 region. IL-33 gene polymorphisms have been reported to be associated with the risk of asthma [10], nasal polyposis [11], inflammatory bowel diseases [12], and Alzheimer's disease [13, 14].

The aim of the present study was to detect the serum IL-33 level and the potential association of IL-33 gene polymorphisms on the susceptibility, in BD.

Materials and methods

Participants

One hundred seventeen unrelated patients with BD, and 149 unrelated healthy controls (HC), from upper Euphrates regions of Turkey, were enrolled in this preliminary candidate gene study. The protocol of this study was approved by the institutional Ethics Committee, and all the participants gave informed consent before enrolling in the study. Detailed histories of all participants were obtained, and their systemic and rheumatological examinations were performed. The pathergy test was performed to all the patients, and 24–48 h later, the patients were evaluated in terms of papulopustular lesions. Patients fulfilled the established criteria [15]. The patients were interpreted as active if those with oral ulcer had at least two of the following pathologies; genital ulcer, skin lesion, recent ocular involvement, recent vascular involvement, recent neurological involvement, active arthritis, positive pathergy test and with high erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP).

Laboratory analysis

Serum IL-33 level was analyzed by *enzyme-linked immunosorbent assay* (ELISA) method using appropriate commercial kit (Bender MedSystems GmbH, Vienna, Austria). In addition, blood samples drawn from all the participants were taken into tubes containing *ethylenediamine tetraacetate* (EDTA) for genotyping. Genomic DNA was immediately isolated from peripheral blood lymphocytes using a commercial kit (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Four single nucleotide polymorphisms (SNPs) of IL-33 gene [rs7044343 (SNP1), rs1157505 (SNP2), rs11792633 (SNP3) and rs1929992 (SNP4)] were genotyped using the primer/probe sets purchased from Qiagen (Hilden, Germany) on *real-time* PCR. These four SNPs were selected since they were evaluated or determined to be related the risks of asthma, nasal polyposis, inflammatory bowel diseases, and Alzheimer's disease in the different ethnic origins by previous studies [10–14].

Statistical analysis

The MedCalc Software version 10.1.6.0 (Mariakerke, Belgium) was used for analysis. Normal distributions were tested with the Kolmogorov–Smirnov test with Lilliefors correction. Quantitative data were presented as mean ± standard deviation (SD). Statistical differences among the groups were identified with *Student's t test*. Genotype frequencies were tested for Hardy–Weinberg equilibrium (HWE), and any deviation between the observed and expected frequencies was tested for significance using the chi-square test. In addition, odds ratio (OR) and 95 % confidence interval (CI) were determined for alleles and haplotype blocks. The Tukey–Kramer's method for multiple testing was used, and p values less than 0.0125 were considered as significant. The linkage disequilibrium and haplotypes were visualized by using the SHEsis software [16].

Results

The demographics

Sixty-five of the healthy volunteers were male while 59 of BD patients were male ($p = 0.327$). The mean ages were 41.8 ± 13.6 and 37.9 ± 10.8 years in the HC and BD groups, respectively ($p = 0.015$). The clinical and laboratory characteristics of the patients with BD were summarized in the Table 1.

Serum IL-33 level

Serum IL-33 level was similar in the BD and HC groups (12.7 ± 8.3 and 14.6 ± 9.5 pg/ml, respectively, and $p = 0.214$). Serum IL-33 levels were 11.3 ± 9.2 and 13.9 ± 7.2 pg/ml in the active BD ($n = 52$) and inactive BD ($n = 65$) subgroups, respectively (Fig. 1). Its level was significantly lower in the active BD subgroup compared to the inactive subgroup and HC group ($p = 0.044$ and $p = 0.037$, respectively).

Serum IL-33 level was significantly higher in patients with uveitis compared to the patients without uveitis (16.4 ± 9.4 vs. 11.3 ± 7.4 pg/ml, $p = 0.028$). However, its level was not significantly different between males and females; with and without any other involvements; using any medications or not ($p > 0.05$ for all). Its level was correlated with only ESR and CRP level ($r = -0.292$, $p = 0.007$ and $r = -0.220$, $p = 0.046$, respectively).

Table 1 The clinical and laboratory characteristics of BD patients

Patients [*n* (%)]	BD ($n = 117$)
Disease duration (years)	5.2 ± 6.2
Hemoglobin (g/dl)	13.6 ± 1.9
WBC count ($10^3/\mu l$)	7.8 ± 3.1
ESR (mm/h)	19.3 ± 19.2
CRP (mg/dl)	14.8 ± 30.6
Active BD [*n* (%)]	52 (44.4)
Oral ulcer [*n* (%)]	117 (100)
Genital ulcer [*n* (%)]	71 (60.3)
Panniculitis [*n* (%)]	53 (45.3)
Vascular involvement [*n* (%)]	18 (15.4)
Uveitis [*n* (%)]	32 (27.4)
Neurological involvement [*n* (%)]	2 (1.7)
Pathergy test positivity [*n* (%)]	18 (15.4)
Acneiform lesions [*n* (%)]	89 (76.1)
Ongoing any medication [*n* (%)]	81 (69.2)
Colchicine [*n* (%)]	73 (62.4)
Azathioprine [*n* (%)]	60 (51.3)
Sulphasalazine [*n* (%)]	10 (8.5)
Cyclosporine [*n* (%)]	5 (4.3)
Methotrexate [*n* (%)]	4 (3.4)
Cyclophosphamide [*n* (%)]	2 (1.7)
Glucocorticoid [*n* (%)]	23 (19.7)

BD Behçet's disease, *WBC* white blood cell, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein

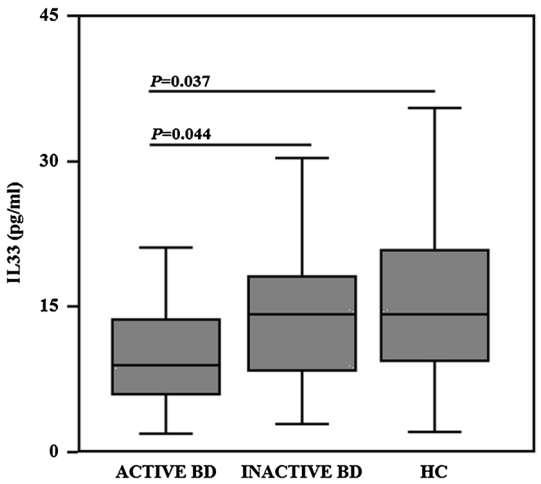

Fig. 1 Serum IL33 levels. *BD* Behçet's disease, *HC* healthy control

IL-33 gene polymorphisms

There was no significant difference in terms of the genotypic and allelic distributions of rs1157505 and rs1929992 polymorphisms between the BD and HC groups (Table 2). However, the frequencies of T allele of both rs7044343 and rs11792633 polymorphisms were significantly lower in the BD group compared to the HC group (Table 2). There were also significant differences in terms of the genotypic distributions of rs7044343 and rs11792633 polymorphisms between the BD and HC groups (Table 2).

CCCA and CCCG haplotypes of IL-33 gene were frequently detected in the BD group than in the HC group ($p = 0.0007$ and $p = 0.002$, respectively). Conversely, CGTA, TCTA and TCTG haplotypes were absent in the BD group (Table 3). The genotypic distributions were in HWE ($p > 0.05$ for all) except for SNP3 in the HC group ($p = 0.0002$). No linkage disequilibrium was found among the four SNPs (SNP1 vs. SNP2: $D' = 0.41$, $r^2 = 0.035$; SNP1 vs. SNP3: $D' = 0.09$, $r^2 = 0.005$; SNP1 vs. SNP4: $D' = 0.48$, $r^2 = 0.169$; SNP2 vs. SNP3: $D' = 0.19$, $r^2 = 0.029$; SNP2 vs. SNP4: $D' = 0.44$, $r^2 = 0.054$; SNP3 vs. SNP4: $D' = 0.06$, $r^2 = 0.002$).

The patients with rs7044343 CT genotype had higher serum IL-33 level compared to the patients with rs7044343 CC genotype (14.8 ± 8.9 vs. 11.1 ± 7.4 pg/ml, $p = 0.036$). Conversely, serum IL-33 level was lower in the patients with rs11792633 CT genotype compared to the patients with CC genotype (9.3 ± 7.6 vs. 14.7 ± 9.1 pg/ml, $p = 0.016$), while ESR (29.8 ± 28.16 vs. 14.2 ± 14.9 mm/h, $p = 0.032$) and CRP level (28.6 ± 45.4 vs. 9.6 ± 23.6 pg/ml, $p = 0.027$) were higher in the former subgroup. The differences of serum IL-33 levels were not significant among the genotypes for all SNPs in the control group (data not shown).

The effects of IL-33 gene polymorphisms on the disease phenotype

The CT variant of rs11792633 and the GG variant of rs1929992 were related with decreased the frequency of uveitis ($p = 0.025$ and $p = 0.030$, respectively). Similarly, the CT variant of rs11792633 seems to protect the patients from vascular involvements ($p = 0.045$). Lower percent of patients with rs1929992 GG genotype was receiving azathioprine ($p = 0.013$). On the other hand, 30.8 % of patients with rs7044343 CT genotype and 12.9 % of patients with CC genotype were receiving glucocorticoid treatments ($p = 0.026$).

Between the males and females, only the genotypic distribution of rs1929992 was fairly different ($p = 0.018$).

Discussion

In the present study, serum IL-33 level and IL-33 gene polymorphisms were evaluated in a cohort of Turkish patients with BD. Serum IL-33 levels were similar in the BD and HC groups. However, its level was lower in the active BD

Table 2 The genotypic and allelic distributions of IL-33 gene polymorphisms

SNPs	Genotypes/alleles	BD ($n = 117$)	HC ($n = 149$)	p and OR (95 % CI)
rs7044343 (C>T)	CC [n (%)]	62 (52.9)	26 (17.4)	$P_{global} < 0.0001$
	CT [n (%)]	54 (46.2)	70 (47.0)	$P_{dominant} < 0.0001$ (OR 0.02, 95 % CI 0.01–0.1)
	TT [n (%)]	1 (0.9)	53 (35.6)	$P_{recessive} < 0.0001$ (OR 0.2, 95 % CI 0.1–0.3)
	C [n (%)]	178 (76.1)	122 (40.9)	<0.0001 (OR 0.2, 95 % CI 0.2–0.3)
	T [n (%)]	56 (23.9)	176 (59.1)	
rs1157505 (C>G)	CC [n (%)]	71 (60.7)	83 (55.7)	$P_{global} = 0.186$
	CG [n (%)]	44 (37.6)	56 (37.6)	$P_{dominant} = 0.070$ (OR 0.2, 95 % CI 0.1–1.1)
	GG [n (%)]	2 (1.7)	10 (6.7)	$P_{recessive} = 0.414$ (OR 0.8, 95 % CI 0.5–1.3)
	C [n (%)]	186 (79.5)	222 (74.5)	0.177 (OR 0.8, 95 % CI 0.5–1.1)
	G [n (%)]	48 (20.5)	76 (25.5)	
rs11792633 (C>T)	CC [n (%)]	84 (71.7)	59 (39.6)	$P_{global} < 0.0001$
	CT [n (%)]	32 (27.4)	45 (30.2)	$P_{dominant} = 0.0001$ (OR 0.02, 95 % CI 0.01–0.2)
	TT n (%)	1 (0.9)	45 (30.2)	$P_{recessive} < 0.0001$ (OR 0.3, 95 % CI 0.1–0.5)
	C [n (%)]	200 (85.5)	163 (54.7)	<0.0001 (OR 0.2, 95 % CI 0.1–0.3)
	T [n (%)]	34 (14.5)	135 (45.3)	
rs1929992 (A>G)	AA n (%)	35 (29.9)	36 (24.2)	$P_{global} = 0.559$
	AG [n (%)]	49 (41.9)	75 (50.3)	$P_{dominant} = 0.621$ (OR 1.1, 95 % CI 0.7–1.9)
	GG [n (%)]	33 (28.2)	38 (25.5)	$P_{recessive} = 0.293$ (OR 0.7, 95 % CI 0.2–1.3)
	A [n (%)]	119 (50.9)	147 (49.3)	0.726 (OR 0.9, 95 % CI 0.7–1.3)
	G [n (%)]	115 (49.1)	151 (50.7)	

IL interleukin, *SNPs* single nucleotide polymorphisms, *BD* Behçet's disease, *HC* healthy control, *OR* odds ratio, *CI* confidence interval

Table 3 The haplotype blocks of IL-33 gene polymorphisms

Haplotypes	BD (%)	HC (%)	p*	OR (95 % CI)
CCCA	43.9	11.1	0.0007	6.67 (3.04–14.64)
CCCG	19.5	4.5	0.002	5.32 (1.72–16.44)
CGTA	–	15.8	0.0001	–
TCTA	–	7.3	0.012	–
TCTG	–	16.6	0.0001	–
CCTA	1.4	7.2	0.069	0.19 (0.03–1.36)
CCTG	3.8	1.9	0.434	2.08 (0.32–13.52)
CGCA	4.7	3.9	0.759	1.26 (0.29–5.42)
TCCA	–	5.8	0.027	–
TCCG	11.7	19.9	0.162	0.55 (0.24–1.28)
TGCA	–	3.4	0.149	–
TGCG	8.4	2.5	0.075	3.69 (0.80–16.99)

BD Behçet's disease, *HC* healthy control, *OR* odds ratio, *CI* confidence interval

* P values of Chi-square test

patients compared to the inactive ones and HC group. In addition, rs7044343 and rs11792633 polymorphisms of IL-33 gene were protective for the susceptibility to the BD in our cohort.

BD is a chronic inflammatory disease. Th1, Th17, regulatory and cytotoxic T cells are the prominent actors of the pathogenesis of BD [2]. Moreover, the local productions and serum levels of several cytokines are exacerbated in BD [2, 7, 17–19]. In particular, Th1-associated cytokines, such as IFN-γ, IL-12, IL-18, and TNF-α, have been found to be increased in patients with BD [2, 17–19]. IL-1 cytokine family cytokines such as IL-1β [20], IL-1 receptor antagonist (IL-Ra) [21], and IL-18 [17, 19] are increased and the genes of IL-1α and IL-1β are reported to be associated with the susceptibility to the BD [22, 23]. IL-33 a novel member of the IL-1 cytokine family is expressed by various types of immune cells such as mast cells, macrophages and dendritic cells, and non-immune cells such as endothelial and epithelial cells [24]. Increased serum level of IL-33 is documented previously in a variety of inflammatory diseases including RA [5–7], systemic sclerosis [8], SLE [9], ankylosing spondylitis [25], inflammatory bowel diseases [12], and multiple sclerosis [7]. However, serum IL-33 level was similar in BD and HC groups in our study. In contrast to previous reports [7–9, 12, 25], its level is documented not to be higher in patients with psoriatic arthritis [5] and dematomyositis/polymyositis [26] those are also inflammatory diseases. Moreover, Mok et al. [27] have infrequently detected serum IL-33 level although Yang et al. [9] are reported higher serum IL-33 level, in SLE patients.

The one cause of the unaltered IL-33 level may be that IL-33 may not act pathogenic roles in the BD. IL-33 is reported to augment the inflammatory process; however, it is believed to be involved in Th2-mediated inflammatory

responses [28]. IL-33 polarizes naïve T cells to Th2, acts as a chemo-attractant for Th2 cells and induces Th2 cytokines such as IL-4, IL-5 and IL-13 [4]. Ben Ahmed et al. [17] have shown that the expressions of IL-4 and IL-13 are not increased in the BD. If so, it is expected that IL-33 should not increase in the BD as in the present study. However, in contrast to our results, Hamzaoui et al. [7] have reported the increased serum IL-33 level in BD. The cause of the discrepancy may be the difference on genetic backgrounds. In our study, IL-33 gene defects were associated with altered serum IL-33 level. Moreover, the difference of clinical involvements in the cohorts may be other cause of the discrepancy. For instance, serum IL-33 levels were higher in patients with eye involvements compared to the patients without this involvement.

In the present study, serum level of IL-33 was lower in the active BD patients compared to the inactive ones, in contrast to the previous article [7]. Previous studies have revealed the increased levels of several cytokines in active disease compared to the quiescent disease [7, 19]. It is expected that the increased cytokines aggravate the production of IL-33 in active BD. Since, a variety of cytokines, including TNF-α and IL-1β those are also associated with BD [20, 29], are documented to enhance the expression of IL-33 [30, 31]. However, it has also been documented that although the each of the TNF-α and IL-17A enhance the expression of IL-33, their concurrent applications do not increase the expression of IL-33 [32]. In addition, it is controversial whether cytokines increase in active BD patients. Or, the sequestration of the cytokines by membrane of inflammatory cells at the time of disease activity may be other cause of decreased IL-33 level. Neely et al. [33] have reported the similar situation for IL-15.

Increased expression of IL-33 has been documented previously by inflammatory and non-inflammatory cells cultured with pro-inflammatory stimuli [30, 31]. On the other hand, nuclear full-length pro-form of IL-33 suppresses pro-inflammatory gene transcription [34]. IL-33 is reported to have a protective effect in atherosclerosis, obesity, type 2 diabetes, and cardiac remodeling [28]. Therefore, it may be also concluded that decreased IL-33 level may be a cause of the disease activity. However, it may also be a bystander. Küchler et al. [35] have reported that IL-33 is expressed by quiescent endothelial cells, but it is not expressed by the same cells in situation of pro-inflammatory stimuli. It is also documented that IL-17 suppresses IL-33 expression by endothelial cells [36].

In the BD, the familial aggregation [37] and the clustering a geographic area that extends along with the ancient *Silk Road* [1] indicate the genetic tendency to the disease. Human leukocyte antigen (HLA)-B51 is the strongest risk factor for BD, and it is confirmed in various ethnic groups [38]. However, the role of the HLA-B51 in the pathogenesis of BD remains to be fully elucidated. In addition, the 32–53 % of BD patients is associated with HLA-B51 in the different ethnic groups [38]. These situations indicate that other genetic factors have to be discovered. Two recent genome wide association studies from Turkey and Japan document that IL-10 and IL23R-IL12RB2 loci are related with the susceptibility [39, 40]. In addition to these cytokines, the different genes of the cytokines including IL-1α, IL-1β, IL-1Ra, IL-6, IL-17, IL-18, TNF-α and TGF-β have been evaluated in several studies documenting controversial results [reviewed in 2].

Gene polymorphisms of IL-1α, IL-1β and IL18 those are IL-1 family cytokines have been reported to related with the disease [22, 23, 41]. IL-33 is a member of IL-1 cytokine member although the coding regions of IL-33 and other members are different. However, in the present study, two SNPs of IL-33 gene were found to be lesser frequent in the BD patients. IL-33 gene polymorphisms are reported to be associated with the increased risk of asthma [10], nasal polyposis [11] and inflammatory bowel diseases [12]. In our study, rs7044343 and rs11792633 variants of IL-33 gene were associated with BD. Similarly, these variants have been related with the decreased risk of Alzheimer's disease and rheumatoid arthritis [13, 14, 42].

Although the functional roles of rs7044343 and rs11792633 those are intronic SNPs are not fully elucidated, it may be concluded that the cause of reduced risk of the disease may be that the polymorphisms of IL-33 gene affect the activity and/or production of IL-33. Altered serum IL-33 levels in the different genotypes in our study support this hypothesis. Moreover, Li et al. [42] have also reported the decreased serum IL-33 level in rheumatoid arthritis patients with rs7044343 CC genotype. However, it is not fully clarified whether IL-33 gene variants affect the susceptibility via suppressing or increasing the activity and level of IL-33.

We realize that the present preliminary study has some limitations. First, the power is below 0.8 for rs1157505 and rs1929992 polymorphisms in view of the fact the minor allele frequencies. However, the powers are above 0.8 for rs7044343 and rs11792633 polymorphism suggesting that sample size is satisfactory for these polymorphisms. Second, HLAB51 accepted the stronger genetic risk factor could be evaluated. Third, other SNPs of IL-33 gene could be evaluated. Moreover, not only IL-33 but also SNPs of other genes surrounding IL-33 gene may be associated with the risk of BD. Lastly, the local expression of IL-33 and sST2L level could be analyzed for more accurate justifying about the association of IL-33 with BD.

Serum IL-33 level is lower in the active BD patients compared to the quiescent patients and healthy subjects. In addition, the rs7044343 and rs11792633 variants of IL-33

gene are associated with the decreased risk of BD in our cohort. In conclusion, it may be suggested that IL-33 may act a protective role on the pathogenesis of BD.

Conflict of interest The authors declare no conflict of interest.

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