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ORIGINAL ARTICLE - GENES AND DISEASE

The IL-33 gene is related to increased susceptibility to systemic sclerosis

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Abstract Systemic sclerosis (SSc) is a chronic inflammatory disease characterized by widespread fibrosis of the skin and several visceral organs. The pro-fibrotic potential of interleukin (IL)-33 has been demonstrated by in both in vitro and in vivo settings; moreover, increased level of IL-33 has also been reported in patients with SSc. Therefore, the aim of the present study was to detect the potential association of IL-33 gene polymorphisms on the susceptibility of SSc. A total of 300 SSc patients and 280 healthy controls (HC) were enrolled in this multicentric preliminary candidate gene study. DNA samples were harvested using an appropriate commercial DNA isolation kit. Four single nucleotide polymorphisms (SNPs) of IL-33 gene (rs7044343, rs1157505, rs11792633 and rs1929992) were genotyped using the appropriate commercial primer/probe sets on *real-time* PCR. There was no significant difference in terms of the allelic distributions and minor allele frequencies of evaluated four IL-33 polymorphisms between the SSc and HC groups ($P > 0.05$ for all). Moreover, the genotypic distributions of rs1157505, rs11792633 and rs1929992 polymorphisms were not significantly different ($P > 0.05$ for all). However, CC genotype of rs7044343 SNP was significantly higher in the SSc group compared to the HC group ($P = 0.013$, OR 1.75, 95 % CI 1.12–2.72). This preliminary candidate gene study demonstrates that rs7044343 polymorphism of IL-33 gene is associated with the susceptibility to the SSc in Turkish population. It may be suggested that IL-33 gene may be a candidate gene to research in SSc.

Keywords Systemic sclerosis · Genetic · IL-33 gene polymorphism

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Introduction

Systemic sclerosis (SSc) is a chronic inflammatory disease characterized by widespread fibrosis of the skin and several visceral organs. The pathogenesis of SSc is not fully known. However, T lymphocytes activate fibroblasts directly via CD154/CD40 ligand or indirectly via pro-fibrotic cytokines including interleukin (IL)-4, IL-6 and transforming growth factor (TGF)-β. Therefore, extracellular matrix components such as collagen and fibronectin are produced by active fibroblasts [1, 2].

Interleukin-33 [IL-1F11 or *nuclear factor from high endothelial venules* (NF-HEV)] is a recently identified cytokine from IL-1 family [3]. ST2, IL-33 receptor, is documented to be expressed by Th2 cells but not by Th1 and Th17 cells [4, 5]. Moreover, recombinant IL-33 applications up-regulate the expressions of IL-5 and IL-13 that are Th2-type cytokines but down-regulate the expression of IFN-γ, a Th1-type cytokine [6]. These results [4–6] suggest that IL-33 is associated with Th2-mediated immunity. IL-4, IL-6 and IL-13 that are Th2-type cytokines stimulates the production of collagen from fibroblasts although Th1-type cytokines including IFN-γ and TNF-α decrease the production of collagen [7]. Moreover, the levels of Th2-type cytokines including IL-4, IL-6, IL-6, IL-10 and IL-13 are documented to be increased in SSc [8, 9], whereas the level of IFN-γ, a Th1-type cytokine, is reported to be decreased in SSc [9]. CD^+ T cells harvested from the skin of SSc patient have documented to show Th2 cytokine profile [10]. These results suggest that Th1/Th2 balance shifts toward Th2 in SSc. The pro-fibrotic potential of IL-33 has been demonstrated by both in vitro and in vivo settings [11]; moreover, increased level of IL-33 has also been reported in patients with SSc [12–14].

Siblings or other first-degree relatives of SSc patients have higher risk (13- to 19-fold increase) for developing SSc [15, 16]. Therefore, over the past few years, the role of genetics in the susceptibility for SSc has been evaluated widely. The established genetic risk factors for SSc are shared among different autoimmune diseases and associated with inflammatory process [21]. IL-33 gene is localized at 9p24 region. IL-33 gene polymorphisms are reported to be associated with allergic rhinitis, Alzheimer's disease, rheumatoid arthritis (RA), ankylosing spondylitis (AS) and Behçet's disease (BD) [17–21]. The aim of the present study was to detect the potential association of IL-33 gene polymorphisms with the susceptibility of SSc.

Materials and methods

Participants

Three hundred unrelated patients with SSc and 280 unrelated healthy controls (HC) from 6 different regions of Turkey were enrolled in this multicentric 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. Patients fulfilled the established criteria [22] and were classified as having diffuse or limited cutaneous SSc. For each patient, the Valentini Disease Activity Index, Medsger Disease Severity Index and modified Rodnan skin score (mRSS) in the SSc group [23, 24] were determined.

Laboratory analysis

At the time of enrollment, antinuclear antibody (ANA), anti-topoisomerase I antibody (ATA) and anti-centromere antibody (ACA) were analyzed using indirect immunofluorescence staining and ELISA method. In addition, blood samples drawn from all the participants were taken into tubes containing *ethylenediaminetetraacetate* (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 to the risks of nasal polyposis, Alzheimer's disease, RA, AS and BD in the different ethnic origins by previous studies [17–21].

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) or median (minimum–maximum). 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 linkage disequilibrium and haplotype blocks were visualized by using the SHEsis software [25]. The Tukey–Kramer's method for multiple testing was used, and P values less than 0.013 were considered as significant.

Results

The demographics

The mean ages were 47.1 ± 12.8 and 44.5 ± 13.8 years in the SSc ($n = 300$) and HC ($n = 280$) groups, respectively ($P = 0.025$). Twenty-eight (9.3 %) of SSc patients and 67 (23.9 %) of healthy volunteers were males ($P < 0.0001$). The clinical and laboratory characteristics

Table 1 Clinical and laboratory characteristics of SSc patients

Clinical and laboratory characteristics	SSc ($n = 300$)
Diffuse cutaneous subtype, *n* (%)	100 (33.3)
Limited cutaneous subtype, *n* (%)	182 (60.7)
ANA positive, *n* (%)	273 (91.0)
ATA positive, *n* (%)	115 (38.3)
ACA positive, *n* (%)	93 (31.0)
IPF, *n* (%)	160 (53.3)
PAH, *n* (%)	46 (15.3)
GI involvement, *n* (%)	61 (20.3)
Renal involvement, *n* (%)	12 (4.0)
Heart involvement, *n* (%)	60 (20.0)
Pitting scar, *n* (%)	141 (47.0)
Flexion deformity, *n* (%)	79 (26.3)

SSc systemic sclerosis, *ANA* anti-nuclear antibody, *ATA* anti-topoisomerase I antibody, *ACA* anti-centromere antibody, *IPF* interstitial pulmonary fibrosis, *PAH* pulmonary arterial hypertension, *GI* gastrointestinal

of the patients with SSc are summarized in Table 1. The duration of the first non-Raynaud's phenomenon symptom was 8.7 ± 6.6 (median 7, min–max 1–40) years, and the disease duration was 5.1 ± 5.6 (median 3, min–max 1–30) years in the SSc group. The mRSS, Valentini Disease Activity Index and Medsger Disease Severity Index were 14.5 ± 7.2, 1.6 ± 0.5, and 5.5 ± 3.1, respectively, in the SSc group.

IL-33 gene polymorphisms

There was no significant difference in terms of the genotypic distributions of evaluated IL-33 polymorphisms between the SSc and HC groups except for rs7044343 (Table 2). CC genotype of rs7044343 SNP was significantly higher in the SSc group compared to the HC group ($P = 0.013$, OR 1.75, 95 % CI 1.12–2.72). However, allelic distributions and MAF of rs7044343, rs1157505, rs11792633 and rs1929992 polymorphisms (Table 2) were not significantly different ($P > 0.05$ for all). The test for HWE showed significant deviations from HWE for SNP1 and SNP4 in the SSc group and for SNP3 in both the SSc and HC groups but not for otherwise.

No linkage disequilibrium was found among the four SNPs (SNP1 vs. SNP2; $D' = 0.44$, $r^2 = 0.097$, SNP1 vs. SNP3; $D' = 0.05$, $r^2 = 0.002$, SNP1 vs. SNP4; $D' = 0.66$, $r^2 = 0.392$, SNP2 vs. SNP3; $D' = 0.09$, $r^2 = 0.002$, SNP2 vs. SNP4; $D' = 0.56$, $r^2 = 0.127$, SNP3 vs. SNP4; $D' = 0.09$, $r^2 = 0.008$). Since there was no significant LD, haplotype analysis was not performed.

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

There was no significant difference in terms of the MAF and the frequencies of genotypic distributions of evaluated SNPs between the diffuse and limited cutaneous subtypes, between patients with and without PAH, pulmonary fibrosis

Table 2 Genotypic distributions and MAF of IL-33 gene polymorphisms in SSc and HC groups

SNPs	Genotypes	SSc ($n = 300$)	HC ($n = 280$)	*P* and OR (95 % CI)
rs7044343 ($C > T$)	CC, *n* (%)	63 (21.0)	37 (13.2)	$P_{global} = 0.026$
	CT, *n* (%)	126 (42.0)	141 (50.4)	$P_{dominant} = 0.013$, OR 0.6 (0.4–0.9)
	TT, *n* (%)	111 (37.0)	102 (36.4)	$P_{recessive} = 0.887$, OR 1.1 (0.7–1.4)
	MAF, *n* (%)	348 (58.0)	345 (61.6)	$P = 0.529$, OR 0.9 (0.8–1.1)
rs1157505 (C > G)	CC, *n* (%)	176 (58.7)	157 (56.1)	$P_{global} = 0.333$
	CG, *n* (%)	99 (33.0)	106 (37.8)	$P_{dominant} = 0.503$, OR 0.9 (0.6–1.2)
	GG, *n* (%)	25 (8.3)	17 (6.1)	$P_{recessive} = 0.296$, OR 1.4 (0.7–2.7)
	MAF, *n* (%)	149 (24.8)	140 (25.0)	$P = 0.959$, OR 0.9 (0.8–1.7)
rs11792633 ($C > T$)	CC, *n* (%)	90 (30.0)	100 (35.7)	$P_{global} = 0.226$
	CT, *n* (%)	124 (41.3)	111 (39.6)	$P_{dominant} = 0.143$, OR 1.3 (0.9–1.8)
	TT, *n* (%)	86 (28.7)	69 (24.7)	$P_{recessive} = 0.274$, OR 1.2 (0.8–1.8)
	MAF, *n* (%)	296 (49.3)	249 (44.5)	$P = 0.097$, OR 1.2 (0.9–1.5)
rs1929992 ($A > G$)	AA, *n* (%)	49 (16.3)	50 (17.9)	$P_{global} = 0.195$
	AG, *n* (%)	177 (59.0)	144 (51.4)	$P_{dominant} = 0.626$, OR 1.1 (0.7–1.7)
	GG, *n* (%)	74 (24.7)	86 (30.7)	$P_{recessive} = 0.104$, OR 0.7 (0.5–1.1)
	MAF, *n* (%)	325 (54.2)	316 (56.4)	$P = 0.678$, OR 0.9 (0.8–1.2)

MAF minor allele frequency, *SSc* systemic sclerosis, *HC* healthy control, *SNPs* single nucleotide polymorphisms, *OR* odds ratio, *CI* confidence interval

$P_{dominant}$: genotype 11 versus genotypes 12 and 22

$P_{recessive}$: genotypes 11 and 12 versus genotype 22

Table 3 Genotypic distributions and MAF of IL-33 gene polymorphisms in the ATA-positive and ATA-negative SSc patients

SNPs	Genotypes	ATA positive ($n = 115$)	ATA negative ($n = 185$)	P and OR (95 % CI)
rs7044343 ($C > T$)	CC, *n* (%)	19 (16.5)	44 (23.8)	$P = 0.017$
	CT, *n* (%)	42 (36.5)	84 (45.4)	
	TT, *n* (%)	54 (47.0)	57 (30.8)	
	MAF, *n* (%)	150 (65.2)	193 (52.2)	$P = 0.001$, OR 1.7 (1.2–2.4)
rs1157505 ($C > G$)	CC, *n* (%)	68 (59.1)	108 (58.4)	$P = 0.041$
	CG, *n* (%)	43 (37.4)	56 (30.3)	
	GG, *n* (%)	4 (3.5)	21 (11.3)	
	MAF, *n* (%)	51 (22.2)	98 (26.5)	$P = 0.235$, OR 0.8 (0.5–1.2)
rs11792633 ($C > T$)	CC, *n* (%)	37 (32.2)	53 (28.6)	$P = 0.242$
	CT, *n* (%)	50 (43.5)	74 (40.0)	
	TT, *n* (%)	28 (24.3)	58 (31.4)	
	MAF, *n* (%)	106 (46.1)	190 (51.4)	$P = 0.210$, OR 0.8 (0.6–1.1)
rs1929992 ($A > G$)	AA, *n* (%)	10 (8.7)	39 (21.1)	$P = 0.012$
	AG, *n* (%)	71 (61.7)	106 (57.3)	
	GG, *n* (%)	34 (29.6)	40 (21.6)	
	MAF, *n* (%)	139 (60.4)	186 (50.3)	$P = 0.015$, OR 1.5 (1.1–2.1)

MAF minor allele frequency, *ATA* anti-topoisomerase I antibody, *SSc* systemic sclerosis, *SNPs* single nucleotide polymorphisms, *OR* odds ratio, *CI* confidence interval

or digital ulcer (data not shown). Similarly, allelic and genotypic frequencies were not significantly different in the ACA-positive ones compared to the negatives. However, the frequencies of CC genotype of SNP1 and AA genotype of SNP4 were lower in the ATA-positive patients than negative ones ($P = 0.017$ and, $P = 0.015$, respectively). The MAF values were 65.2 and 52.2 % for SNP1 ($P = 0.001$, OR 1.7, 95 % CI 1.2–2.4) and 60.4 and 50.3 % for SNP4 ($P = 0.012$, OR 1.5, 95 % CI 1.1–2.1), in the ATA positives and negatives, respectively, and were significantly higher in the former group (Table 3). However, the distributions of alleles and genotypes of SNP2 and SNP3 were not significantly different between the ATA positives and negatives.

The female proportion was significantly greater in the SSc group than in HC group ($P < 0.001$). However, the distributions of alleles and genotypes were not significantly different between the females and males in the both groups.

Discussion

The present study demonstrated that there was no significant difference for allelic distributions and MAF values of rs7044343, rs1157505, rs11792633 and rs1929992 polymorphisms of IL-33 gene between the SSc patients and healthy controls. However, the genotypic distributions of rs7044343 SNP were significantly different in the SSc and HC groups. The CC genotype of rs7044343 SNP was related to the increased susceptibility to SSc in our cohort.

The pathogenesis of SSc consists of a triad including fibrosis, vasculopathy and inflammation. Fibrosis occurs as a result of excessive ECM production or insufficient ECM degradation. Fibroblasts, which are responsible for the production of ECM, are the main actors in the pathogenesis of SSc. In this process, the pro-fibrotic cytokines and chemokines are responsible for the transformation and proliferation of activated fibroblasts [26, 27]. These mediators are released from inflammatory cells and activated and/or damaged endothelial cells. It is known that the inflammation and vasculopathy as pathogenic stages of SSc begin the adventure before fibrosis. The infiltration of inflammatory cells such as T lymphocytes, mast cells and macrophages has been shown in the skin biopsies of patients with SSc [26, 27].

Interleukin-33, a cytokine, has prominent roles on the pathogenesis of inflammatory diseases, cancer and atopic, cardiovascular and central nervous system disorders. In addition to endothelial and epithelial cells, it is also produced by fibroblasts, innate lymphoid and T cells [28, 29]. It has been clarified that IL-33 binds a heteromeric receptor complex. Its receptor has subunits that are the orphan IL-1 receptor (ST2), and IL-1R accessory protein (IL-1RAcP) [3, 30]. IL-33 binds this complex and thus induces the activation of NF-kappaB and MAP-kinases, leading to IL-4, IL-5 and IL-13 productions [3]. IL-4 and IL-13 are the most potent effectors of fibrosis in SSc [1, 2]. Previous studies [12–14] showed that serum concentration of IL-33 and tissue expression of ST2L were elevated in SSc patients.

Since IL-33 is one of the pathogenic actors in the etiopathogenesis of SSc, it is realized that IL-33 gene is a candidate to research in SSc. Moreover, recent genetic studies [17–21] have demonstrated that several polymorphisms of IL-33 gene are associated with different inflammatory disorders and immune-related conditions. We also showed that the C allele and CC genotype of rs7044343 gene are associated with the increased risk of BD [21]. Similarly, CC genotype of rs7044343 was reported to be decreased frequency in Chinese RA patients [19]. In the present study, the rs7044343 SNP of IL-33 gene is associated with susceptibility to SSc.

These results suggest that IL-33 gene is a candidate to research in SSc. Moreover, the AA genotype of rs1929992 was rare in the ATA-positive patients compared to the negatives (OR 0.36, 95 % CI 0.17–0.75), in our study. Moreover, the MAF values of rs7044343 and rs1929992 were higher in the former subgroups. Namely, these SNPs affect disease subsets. IL-33 gene polymorphism can also affect inflammatory ways. Li et al. [19] showed that rs7044343 SNP of IL-33 gene alters serum IL-33 level in patients with RA. Moreover, Luo et al. [31] reported that an IL-33 polymorphism modifies eosinophilia in rats.

It is known that the family history of SSc increases the risk of developing disease [15, 16]. Genetic is accepted to have prominent role on the etiopathogenesis of SSc [32]. Recent genome-wide association studies (GWAS) have advanced our knowledge about the genetic basis of SSc [33–35]. However, SNPs located within IL-33 gene have not been associated with SSc in these comprehensive studies [33–35]. According to these GWAS result, the number of detected loci explaining the genetic component of SSc is limited. Although candidate gene studies have several limitations compared to GWAS, they have also several advantages. For instance, several genetic loci are documented by candidate gene study, but they are not observed by GWAS [reviewed in 32].

We realize that the present study has some limitations. First, power analysis was not performed before entering the participants in the study. However, the power is 0.78 for rs7044343 SNP, suggesting that sample size is satisfactory for this polymorphism. The powers of other SNPs were below 0.6, suggesting that their negative associations need to be corrected. Second, 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 SSc. Further studies may be required to elucidate causal polymorphisms of the detected associations.

In conclusion, this preliminary candidate gene study demonstrates that rs7044343 SNP of IL-33 gene is associated with the susceptibility to the SSc in Turkish population. It may be suggested that IL-33 gene may be a candidate gene to research in SSc.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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