Rheumatol Int (2009) 29:921–925
DOI 10.1007/s00296-008-0776-4

ORIGINAL ARTICLE

Genetic variation in the interleukin-10 gene promoter in Polish patients with systemic lupus erythematosus

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Received: 29 July 2008 / Accepted: 9 November 2008 / Published online: 10 December 2008
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Abstract Identification of susceptibility genes in systemic lupus erythematosus (SLE) has recently become a topic of interest. The IL-10 promoter contains three single base-pair substitutions at −627C > A, −854C > T and −1117G > A. These single base-pair substitutions produce three different haplotypes, GCC, ACC and ATA, which affect IL-10 expression. We examined the distribution of −627C > A, −854C > T and −1117G > A IL-10 promoter polymorphisms in patients with SLE ($n = 103$, women only) and matched controls ($n = 300$). Despite the higher prevalence of the GCC/GCC, GCC/ATA and ATA/ATA genotypes in SLE patients than in controls, we observed that only GCC/GCC genotype frequency distribution was significant between these groups. We observed that women with the GCC/GCC genotype displayed an approximately twofold increased risk of SLE OR = 2.245 (95% CI = 1.354–3.721, $P = 0.0022$). We did not find any associations between various genotypes of IL-10 promoter haplotypes and clinical manifestations or autoantibody production in patients with SLE. Our observations indicate that the GCC/GCC promoter genotype may contribute to SLE incidence in Polish patients.

Keywords Systemic lupus erythematosus · IL-10 · Promoter polymorphisms

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that affects virtually every organ [1, 2]. SLE is accompanied by an abundant production of antibodies directed against self-antigens and deposition of immune complexes in multiple organs [1, 2]. Immune cells from patients with SLE display various aberrations including abnormal cytokine production, reduction of cytotoxic T cell function and enhancement of humoral response [3, 4].

Despite intensive research in recent years, the etiology of SLE is still unknown. The identification of susceptibility genes has recently become a hot topic. Many genes encoding proteins relevant to the immune system or genes encoding proteins affecting SLE manifestations have been linked to the disease as candidate susceptibility genes [5–9].

It has been reported that interleukin 10 (IL-10) is biosynthesized in high amounts in the B cells and monocytes of patients with SLE [10]. Moreover, the abundant biosynthesis of IL-10 affects the abnormal biosynthesis of autoantibodies that occurs in patients with SLE [10]. IL-10 is an anti-inflammatory cytokine that inhibits the production of proinflammatory cytokines in activated macrophages [11].

It has been reported that IL-10 biosynthesis is regulated at a transcriptional level; so, polymorphic forms of the promoter may alter binding transcription factors and affect the activity of the IL-10 promoter [12–14]. The IL-10 promoter contains three single base-pair substitutions at −627C > A, −854C > T and −1117G > A, which produce the three different haplotypes GCC, ACC and ATA [13, 15]. The haplotypes GCC, ACC and ATA were associated with high, intermediate and low IL-10 production, respectively, according to studies of this promoter's variants [16, 17].

Numerous investigations have been undertaken to determine the association of different IL-10 promoter variants with SLE. Moreover, contribution of the IL-10 promoter variants in the development of SLE and the promoter's association to several clinical features have been inconsistent [18–29]. We examined the distribution of −627C > A, −854C > T and −1117G > A IL-10 promoter polymorphic variants in Polish patients with SLE.

Patients and methods

Patients and controls

One hundred and three patients (women only) fulfilling the American College of Rheumatology Classification (ACRC) criteria for SLE [30, 31] were chosen for investigation at the Institute of Rheumatology, Warsaw, Poland. The controls included 300 healthy women. No male patients were available for the study. The protocol of the study was approved by the Local Ethical Committee of Poznań University of Medical Sciences. Written consent was obtained from all participating subjects. Both patients and control groups were of Polish Caucasian origin. The mean age of patients with SLE at diagnosis was 37 ± 12 years, and of controls, 34 ± 13 years.

Clinical symptoms in the patient group are found in the central nervous system (20%), as well as having vascular (13%), renal (51%), musculoskeletal (63%), serosal (17%), dermal (54%), immunologic (24%), constitutional (fever) (10%) and hematologic (29%) components.

Genotyping

DNA was isolated from peripheral blood lymphocytes by salt extraction. Polymorphic variants of −627 IL-10 promoter were identified using PCR and the primer pairs 5′ACCTGACTAGCATATAAGAAGC3′ and 5′AGCCAC AATCAAGGTTTCCC3′; enzyme digestion followed the identification process [18]. The PCR-amplified fragments of the IL-10 promoter that were 669 base pairs (bp) in length were subjected to digestion with *RsaI* (GT/AC). The −627A allele was cleaved into 503-bp and 166-bp fragments, whereas the −627C allele remained uncut. DNA fragments were separated by electrophoresis on 2.5% agarose gel and visualized by ethidium bromide staining [18]. Confirmation of −627C > A polymorphism was performed by sequencing analysis. The 854C > T and −1117G > A IL-10 promoter polymorphic variants were identified using PCR with the primer pair 5′AACACTCCTCGCC GCAACC3′ and 5′CCCCTACCGTCTCTAT TTTAT3′, followed by direct sequencing analysis of PCR-amplified IL-10 promoter fragments of 430 bp.

Statistical analysis

The distribution of genotypes in all groups was tested for deviation from Hardy–Weinberg equilibrium. Fisher exact test was used to determine differences in the genotypic and allelic distribution between patients and controls. Moreover, the odds ratio (OR) and 95% confidence intervals (CI) were calculated. A P value <0.05 was considered statistically significant. Power analysis was performed using uncorrected chi-square test available from an on-line internet service, http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize.

Results

Distribution of −627C > A, −854C > T and −1117G > A IL-10 promoter polymorphisms in patients with SLE

Genotype analysis of IL-10 promoter polymorphisms did not show a significant deviation from Hardy–Weinberg equilibrium in any group. The frequency of the −627A and −854T alleles was higher in patients with SLE compared to controls and reached 26 and 24%, respectively, in these groups (Table 1). The homozygous −627CC and −854CC genotype frequency was lower in patients than in controls (Table 1). However, the frequency of the homozygous −627AA and −854TT genotypes was slightly higher in patients with SLE (Table 1).

The −1117G IL-10 allele frequency was significantly higher in patients than in controls ($P = 0.0035$) and amounted 55 and 43%, respectively (Table 1). We also found a higher distribution of homozygous −1117GG in patients compared to controls. The frequency of homozygous −1117GG reached 33 and 18% in these groups, respectively (Table 1). However, the heterozygous −1117GA and homozygous −1117AA prevalence was lower in patients than in controls (Table 1).

Genotype distribution of promoter's haplotype in patients with SLE and controls

The genotypes GCC/GCC, GCC/ATA and ATA/ATA exhibited higher prevalence in patients with SLE than in healthy individuals (Table 2). However, only the GCC/GCC genotype frequency distribution was significant between these groups (Table 2). OR for patients with SLE with the GCC/GCC genotype was OR = 2.245 (95% CI = 1.354–3.721, $P = 0.0022$) (Table 2). We did not find any associations between various genotypes of the IL-10 promoter haplotype with clinical manifestations or autoantibody production in patients with SLE. The statistical power of this study amounted to 87% for GCC/GCC genotypes.

Table 1 Distribution of the −627C > A, −854C > T and −1117G > A IL-10 promoter polymorphisms in SLE patients and control

	Number (*n*)	Genotype distribution absolute number (frequency %)			Alleles absolute number (frequency %)		
		−627C/C	−627A/C	−627A/A	−627C	−627A	P^a
IL10 −627C > A	Controls 300 Total (females)	177 (59)	102 (34)	21 (7)	456 (76)	144 (24)	0.6389
	SLE 103 Total (females)	59 (57)	35 (34)	9 (9)	153 (74)	53 (26)	
	Number (*n*)	Genotype distribution absolute number (frequency %)			Alleles absolute number (frequency %)		
		−854C/C	−854C/T	−854T/T	−854C	−854T	P^a
IL10 854C > T	Controls 300 Total (females)	177 (59)	102 (34)	21 (7)	456 (76)	144 (24)	0.6389
	SLE 103 Total (females)	59 (57)	35 (34)	9 (9)	153 (74)	53 (26)	
	Number (*n*)	Genotype distribution absolute number (frequency %)			Alleles absolute number (frequency %)		
		−1117G/G	−1117G/A	−1117A/A	−1117G	−1117A	P^a
IL10 −1117G > A	Controls 300 Total (females)	54 (18)	150 (50)	96 (32)	258 (43)	342 (57)	0.0035
	SLE 103 Total (females)	34 (33)	45 (44)	24 (23)	113 (55)	93 (45)	

[a] Fisher exact test

Table 2 Genotype distribution of three promoter haplotypes in patients with SLE and controls

Genotype	Controls absolute number (frequency %)	SLE absolute number (frequency %)	Odds ratio (95% CI), P^a
GCC/GCC	54 (18)	34 (33)	2.245 (95% CI 1.354–3.721)[b], $P = 0.0022$
GCC/ACC	90 (30)	23 (22)	
GCC/ATA	60 (20)	22 (21)	
ACC/ACC	33 (11)	2 (2)	
ACC/ATA	42 (14)	13 (13)	
ATA/ATA	21 (7)	9 (9)	

[a] Fisher exact test; the odds ratio was calculated for patients

[b] GCC/GCC genotype versus other genotypes

Discussion

IL-10 is a significant cytokine that suppresses both immunoproliferative and inflammatory responses [32]. The IL-10 gene is localized to the junction of 1q31–q32 [15]. IL-10 is a 36-kDa homodimeric cytokine and is mainly biosynthesized by macrophages, monocytes and lymphocytes [10, 32]. It has been reported that IL-10 production after lipopolysaccharide whole blood stimulation differs significantly between humans as a result of genetic variations acting at the transcriptional level [12, 33].

The nucleotides A and G are present at the IL-10 promoter in similar frequencies at position −1,117, while a C → A substitution at position −627 is present in 21–23% of the population and is in complete linkage disequilibrium with C → T at position −854 [13, 15]. Investigation of IL-10 expression in various diseases revealed an increased IL-10 production in individuals bearing the GCC haplotype [12, 16, 34]. Transient transfection studies have also confirmed higher transcriptional promoter activity for the GCC haplotype as compared to the ATA IL-10 promoter haplotype [17].

We observed that the homozygous GCC haplotype increased the risk of SLE development approximately twofold; however, we did not observe an association of this variant or other promoter variants with clinical manifestations of SLE or with autoantibody production.

Our results are different from findings in other populations showing lack of promoter variant association with SLE incidence or ATA haplotype contribution to SLE

[18, 20–24, 35, 36]. Lin et al. reported that the −627A IL-10 genotype and allelic frequency was significantly increased in patients with SLE compared to controls [18]. In Chinese patients, the ATA haplotype was associated with kidney disorders but not with autoantibody production [24]. Rood et al. observed a significant association between the ATA haplotype and neuropsychiatric manifestations in patients with SLE [23].

However, our results were consistent with observations in other investigations suggesting GCC haplotype contribution to SLE [19, 26, 28, 29]. Khoa et al. found that the allele frequency of −1117G in patients with SLE was significantly higher than that in healthy controls [19]. Morover, Chung et al. found that the homozygous GCC haplotype was associated with greater SLE severity [28]. Recently, Rosado et al. have suggested that the IL-10 promoter haplotype that biosynthesizes higher levels of IL-10 is linked with the severity of SLE in patients [29]. Lazarus et al. observed that only the IL-10 promoter haplotype GCC was linked with renal disease and Ro autoantibody production in Caucasian patients with SLE [25].

Since the GCC haplotype of the IL-10 promoter is associated with high transcriptional activity [12, 15, 17], we can assume that a high production of IL-10 may make an individual more susceptible to an abundant production of autoantibodies during inflammatory processes, which may favor SLE incidence.

The differences in the effects of IL-10 promoter variants on incidence or clinical manifestations of SLE may be due to distinct racial structure and environmental factors acting on investigated populations. It is possible that different polymorphisms in linkage disequilibrium with the investigated IL-10 promoter variant may contribute to SLE incidence. To determine more precisely the associations of the IL-10 promoter genotype with patients with SLE, further examination of these variants' distribution in other populations are needed.

Acknowledgments This study was supported by Poznan University of Medical Sciences (grant number 502-01-01124182-07474).

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