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Interleukin-7 receptor Thr244Ile gene polymorphism and the risk of systemic lupus erythematosus

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ABSTRACT

Aim. Recently, the *IL-7 receptor* (*IL-7R*) C>T (rs6897932) single nucleotide polymorphism (SNP), which causes a Thr244Ile substitution in the IL-7R α-chain, has been suggested as a risk factor for SLE.
Material and Methods. Using high-resolution melting curve analysis we studied the distribution of the *IL-7R* C>T polymorphism in SLE patients (n = 281) and control subjects (n = 541) in the Polish population.
Results. We did not find significant differences in the distribution of the *IL-7R* C>T genotype and alleles between SLE patients and controls. However, in the dominant model (T/T and C/T vs C/C genotypes), we observed a protective effect of the *IL-7R* C>T polymorphism against the presence of neurological manifestations of SLE [OR = 0.3631 (95% CI = 0.1895–0.6954), p = 0.0017, p_{corr} = 0.0323] and the presence of anti-Scl-70 antibodies (Ab) [OR = 0.3141 (95% CI = 0.1503–0.6561), p = 0.0014, p_{corr} = 0.0266].
Conclusion. Our studies suggest that the *IL-7R* C>T (rs6897932) polymorphism might be involved in the neurological manifestations and the presence of anti-Scl-70 Abs in patients with SLE.

Keywords: Interleukin-7 receptor, SNP, SLE.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder in which the immune system of the host attacks its own tissues [1]. The SLE immune cells are characterized by abnormal signaling in $CD4^+$ T cells as well as abundant autoantibody biosynthesis by B cells [1–3]. This disease can affect the kidneys, joints, skin, lungs, brain, and other organ systems, resulting in defective functioning of organs, as observed in clinical findings of SLE [1]. Familial and genome-wide association studies have suggested many genes that potentially play a role in SLE development, phenotypes and antibody profiles. Exposure to various exogenous factors such as ultraviolet light, drugs, chemicals, pollutants, and bacterial and viral infections all contribute to SLE development. The underlying cause of SLE remains unknown; however, it is accepted that the genetic components of the host and environmental factors make the host vulnerable to this autoimmune disorder [4, 5].

Recently, an increased body of evidence has demonstrated an association of abnormal interleukin-7 (IL-7) signaling with aberrant functions of immune cells and autoimmunity [9]. IL-7 signaling plays an elementary role in B lymphopoiesis, thymocyte maturation, peripheral T cell homeostasis and immune tolerance [10–12]. IL-7 receptor (IL-7R) is a heterodimer comprising IL-7Rα and the common γ-chain, which is also shared by IL-2R, IL-4R, IL-9R, IL-15R, and IL-21R [13, 14]. The *IL-7R* C>T (rs6897932) polymorphism causes a Thr244Ile substitution in the IL-7R α-chain, thereby changing the ratio of membrane-bound to soluble IL-7R, which is implicated in the pathogenesis of autoimmune diseases [15,

16]. It has been demonstrated that the Thr244Ile substitution can be associated with some autoimmune diseases [15, 17–19]. Recently, the *IL-7R* C>T SNP has also been recognized as a risk factor for SLE development [18]. Therefore, we evaluated whether the *IL-R7* C>T SNP is a genetic risk factor for SLE in the Polish population. Because SLE is a heterogeneous disorder, we also examined the association of this polymorphism with different disease phenotypes and antibody profiles.

Material and Methods

Patients and controls

Medical records data for two hundred and eighty-one women fulfilling the American College of Rheumatology Classification criteria for SLE were collected for the study in a random manner at the Institute of Rheumatology in Warsaw, Poland [20, 21]. The control group comprised five hundred and forty-one unrelated healthy female volunteers that were selected during medical examination at the Institute of Mother and Child in Warsaw, Poland. The women with SLE and the controls were of Polish Caucasian origin and of similar age. The mean age was 37 ± 8 years for the SLE patients at diagnosis and 36 ± 7 years for the controls. All participating subjects provided written consent. The study procedures were approved by the Local Ethical Committee of Poznan University of Medical Sciences in Poznan, Poland.

Genotyping

DNA was isolated from peripheral leukocytes using a salting-out procedure. The *IL-7R* C > T (rs6897932) DNA fragment (135bp) was amplified using the primers 5′ TGAGACCCTACCCCCACT 3′ and 5′ GCCAAGATGACCAACAGAG 3′. This polymorphism was then genotyped by high-resolution melting curve analysis (HRM) on a Light Cycler *480* system (Roche Diagnostics, Mannheim, Germany). The *IL-7R* C>T polymorphisms were verified by commercial sequencing analysis.

Statistical analysis

The prevalence of genotypes in patients and controls was examined for deviation from Hardy-Weinberg equilibrium using exact and log likelihood ratio chi-squared (χ^2) tests [http://ihg.gsf.de/cgi-bin/hw/hwa1.pl]. The polymorphism was tested for association with the SLE incidence using the χ^2 test for trend (p_{trend}). The χ^2 test was employed to examine differences in genotypic and allelic distribution between patients and controls, and a p value <0.05 was considered statistically significant. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. The association of the *IL-7 receptor* (*IL-7R*) C>T SNP polymorphism with clinical manifestations and the presence of autoantibodies was evaluated by χ^2 test. The Bonferroni correction for multiple comparisons was used, and both p values, before (p) and after correction (p_{corr}), were evaluated.

Results

Prevalence of *IL-7R* C>T (rs6897932) genotypes and alleles in SLE patients and controls.

The genotypic prevalence of the *IL-7R* C>T polymorphism did not significantly deviate from Hardy-Weinberg equilibrium between patients with SLE and healthy controls. The number of genotypes and the ORs and 95% CIs for the *IL-7R* C>T SNP are listed in **Table 1**. We did not observe association of *IL-7R* C>T *SNP* with SLE development. The OR for SLE patients with the *IL-7R* TT genotype was 0.5906 (95% CI = 0.2927–1.192, p = 0.1378) the OR for the CT genotype was 1.008 (95% CI = 0.7401–1.373, p = 0.9602), the OR for the TT and C/T genotypes was 0.9397 (95% CI = 0.6988–1.264, p = 0.6803), and the OR for the *C* allele was

Table 1. Prevalence of the *IL-7R* C>T (rs6897932) polymorphism in SLE patients and controls

IL-7R **C>T**	SLE n = 281	Controls n = 541	OR	95%CI	P-value[e]	P_{trend}
Genotype frequency						
C/C	174 (0.62)	327 (0.61)	**Reference**			
C/T	96 (0.34)	179 (0.33)	1.008[a]	(0.7401–1.373)	0.9602[a]	
T/T	11 (0.04)	35 (0.06)	0.5906[b]	(0.2927–1.192)[b]	0.1378[b]	0.3600
C/T + T/T	107 (0.38)	214 (0.39)	0.9397[c]	(0.6988–1.264)[c]	0.6803[c]	
Minor allele frequency						
T	0.21	0.23	0.8891[d]	0.6941–1.139[d]	0.3517[d]	

The Odds Ratio (OR) was calculated for patients [a](C/T vs C/C genotype), [b](T/T vs C/C genotype),[c] (T/T and C/T vs **C/C genotype). We also determined the OR for the patients' minor allele**; [d](T allele vs C allele); [e]χ^2 test.

0.8891 (95% CI = 0.6941–1.139, p = 0.3517). The p value of the χ^2 test for the trend observed for the *IL-7R* C>T polymorphism was also not statistically significant (p_{trend} = 0.3600).

Association of the *IL-7R* C>T SNP with the presence of autoantibodies and clinical manifestations in patients with SLE.

In the dominant model (T/T and C/T vs C/C genotype), we observed a significant protective effect of the *IL-7R* C>T polymorphism against the presence of neurological manifestations of SLE [OR = 0.3631 (95% CI = 0.1895–0.6954), p = 0.0017, p_{corr} = 0.0323] (**Table 2**). We also found a statistically significant protective effect of the *IL-7R* C>T SNP against the presence of anti-Scl-70 Abs [OR = 0.3141 (95% CI = 0.1503–0.6561), p = 0.0014, p_{corr} = 0.0266] (**Table 3**). However, we did not find any significant differences between the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) at diagnosis and the *IL-7R* C>T genotypes.

Discussion

Abnormal concentrations of IL-7R and IL-7Rα on T cells have been demonstrated in blood plasma from patients with SLE [22–24]. The IL-7R levels were significantly higher in SLE patients than in controls and correlated with SLEDAI scores, especially nephritis [22]. In addition to this finding, Kim [23] demonstrated increased levels of IL-7Rα in low effector memory $CD8^+$ T cells, which may affect tissue damage via CD244-mediated cytotoxicity in patients with SLE. Furthermore, Wang [24], using a mouse model of SLE-like serology, found that the function of IL-7R was required for reintroducing RAG proteins into antigen-activated early memory plasma B cells or pre-plasma B cells and contributed to the maintenance of humoral tolerance. Therefore, the genetic variants of *IL-7R*α might influence different SLE phenotypes and antibody profiles.

In conclusion, we did not observe a contribution of the *IL-7R* C>T polymorphism to SLE development in the

Table 2. Distribution of the *IL-7R* C>T (rs6897932) polymorphism among SLE patients with different clinical manifestations

Characteristic	Genotype distribution				MAF[d]	
	C/C SLE[a] / SLE[b]	C/T SLE[a] / SLE[b]	T/T SLE[a] / SLE[b]	**Odds ratio (95% CI), p[c]** T/T + C/T vs C/C	SLE[a]	SLE[b]
Malar rash	91 / 83	49 / 47	6 / 5		0.21	0.21
Discoid rash	52 / 122	28 / 68	4 / 7		0.21	0.20
Phototosensitivity	79 / 95	43 / 53	7 / 4		0.22	0.20
Oral or nasopharyngeal	67 / 107	39 / 57	5 / 6		0.22	0.20
Arthritis	39 / 135	22 / 74	3 / 8		0.22	0.21
Serositis	31 / 143	17 / 79	2 / 9		0.21	0.21
Renal	84 / 90	46 / 50	7 / 4		0.22	0.20
Neurologic	51 / 123	12 / 84	2 / 9	0.3631 (0.1895–0.6954), p = 0.0017	0.12	0.24
Hematologic	56 / 118	30 / 66	5 / 6		0.22	0.21
Immunologic	84 / 90	43 / 53	10 / 1		0.23	0.19
ANA	174 / 174	96 / 96	11 / 11			

Comparison of genotype frequencies between patients (SLE[a]) with and patients (SLE[b]) without a particular manifestation was performed by [c]χ^2 test, minor allele frequency[d].

Table 3. Effect of the *IL-7R* C>T (rs6897932) polymorphism on the presence of various autoantibodies in patients with SLE

Autoantibodies	Genotype distribution				MAF[d]	
	C/C SLE[a] / SLE[b]	T/C SLE[a] / SLE[b]	T/T SLE[a] / SLE[b]	Odds Ratio (95% CI)[a], p[c] T/T and T/C vs C/C	SLE[a]	SLE[b]
anti-dsDNA	58 / 116	31 / 65	7 / 4		0.23	0.20
anti-Smith	15 / 159	8 / 88	2 / 9		0.24	0.21
anti-snRNP	33 / 141	17 / 79	6 / 5		0.26	0.20
anti-Ro	28 / 146	15 / 81	3 / 8		0.23	0.21
anti-La	23 / 151	12 / 84	3 / 8		0.24	0.21
anti-Scl-70	43 / 131	7 / 89	3 / 8	0.3141 (0.1503–0.6561), p = 0.0014	0.12	0.23

Comparison of genotype frequencies between patients (SLE[a]) with and patients (SLE[b]) without an autoantibody was performed by [c]χ^2 test.
minor allele frequency[d].

Polish population. Our results were contradictory to the findings of Wang [18], who demonstrated the IL-*7R* C gene variant as a risk factor for SLE in their studied Chinese population. However, in our study we observed a significant association between the IL-*7R* C>T polymorphism and the presence of neurologic manifestations in patients with SLE and the presence of anti-Scl-70 Abs. In contrast, Wang [18] did not observe an association of this SNP with any clinical features of SLE.

The IL-*7R* C gene variant has been demonstrated as a risk factor for multiple sclerosis (MS), type I diabetes (T1D), chronic inflammatory arthropathies and atopic dermatitis [15, 17, 19, 25, 26]. The other SNP, rs10213865, being in complete linkage with *IL-7R* C>T, has been associated with sarcoidosis [27]. The IL-*7R* C>T polymorphism has also been associated with the risk of hematopoietic cell transplantation relapse in patients with hematological malignancies, and with mortality among untreated HIV-infected Zimbabwean individuals [28, 29]. Moreover, other genetic variations in *IL-7R* are implicated in inhalation allergy, Omenn syndrome (MIM 603554), graft-versus host disease, inflammatory bowel disease and primary biliary cirrhosis [30–34].

The role of the *IL-7R* C>T polymorphism in the development of autoimmunity has been evaluated in some studies [15, 16, 35–37]. Gregory [15] demonstrated that this polymorphism is situated inside of the alternatively spliced exon 6 of *IL-7R* and disrupts an exonic splicing silencer, which alters the ratio of soluble and membrane-bound IL-7R isoforms. McKay [35] demonstrated that two *IL-7R* haplotypes having the *IL-7R C>T* SNP contributed to the levels of mRNA encoding the sIL-7R isoforms. McKay [35] also showed that this MS susceptibility haplotype was accompanied by the over-presentation of sIL-7R isoforms in the peripheral blood of patients with primary progressive MS. These findings were confirmed by Lundström [16], who observed that individuals with MS with the IL7R CC genotype displayed an increased level of circulating sIL-7Rα. They also demonstrated that sIL-7Rα potentiates IL-7 bioactivity, contributing to the increased risk of autoimmunity in subjects with a genotype linked to heightened sIL7Rα [16]. The sIL-7Rα levels also correlated with the IL-*7R* C risk allele in patients with T1D [36]. Recently, Kreft [37] demonstrated that sIL-7Rα levels corresponded to the *IL-7R* C risk allele and abnormal IL-7; therefore, the IL-7Rα concentration may influence the responsiveness of IL-7Rα^{+} T cells.

In conclusion, our study suggests that the *IL-7R* T gene variant may protect against neurological manifestations of SLE and the presence of anti-Scl-70 Abs. However, to confirm the role of the *IL-7R* C>T SNP in SLE, similar studies should be conducted with larger samples of different ethnicities.

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Conflict of interest statement
The authors declare no conflict of interest.

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