Clinical and Experimental Rheumatology 2011; 29: 310-313.

Interleukin-18 promoter polymorphisms in patients with systemic lupus erythematosus

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Supported by a grant no. 502-01-01124182-07474, from Poznań University of Medical Sciences.

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Received on July 1, 2010; accepted in revised form on December 15, 2010.

Key words: systemic lupus erythematosus, *IL-18*, polymorphisms

Competing interests: none declared.

ABSTRACT

Objectives. *The role of various polymorphisms located in the* IL-18 *promoter has not yet been defined with regards to patient susceptibility to SLE, and occurrence of clinical manifestations of the disease remains inconsistent.*

Methods. *Using PCR-RFLP and DNA sequencing analysis we studied the frequency of -137 G/C (rs187238), -607 C/A (rs1946518) and -1297C/T (rs360719) polymorphisms in IL-18 promoter in patients with SLE from a sample of the Polish population.*

Results. *We observed that patients with SLE bearing the IL-18 -1297CC genotype exhibited a 2.536-fold increased risk of SLE incidence (95% CI=1.333–4.826, p=0.0035). We also found a significantly higher frequency of the IL-18 -1297C allele in patients than in controls, with odds ratio (OR) for the IL-18 -1297C allele in patients with SLE being 1.558 (95% CI=1.189–2.041, p=0.0013). Moreover, there was an association between the IL-18 -1297CC genotype and renal manifestations of SLE, OR=3.792 (1.446–9.947, p=0.0051). However, we did not find any contribution of the IL-18 -607 C/A and -137 G/C polymorphisms to SLE incidence or occurrence of the studied SLE manifestations.*

Conclusions. *Our findings confirmed that the IL-18 -1297C gene variant may contribute to the risk of SLE incidence. Moreover, IL-18 -1297CC might be associated with renal manifestations of SLE.*

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder that affects many human tissues and organs (1). The immune system mounts an immune response against the host body's own tissues and organs, leading to their inflammation, damage, and dysfunction (1). Individuals with SLE exhibit a reduced action of cytotoxic T cells, aberrant function of $CD4^+$ T cells, abnormal activation of B cells, and skewed cytokine biosynthesis (1). The flare-ups of this autoimmune disease can be triggered by various environmental factors, chemicals and infectious pathogens (1). Moreover, genetic studies have shown a strong contribution of many gene variants to SLE incidence and clinical symptoms (2-4).

Interleukin-18 (*IL-18*) is a proinflammatory cytokine, which affects the innate and acquired immune response (5). *IL-18* is produced by various immune cells and displays numerous biological functions, including a pleiotropic impact on Th1 and Th2 type immune responses (5). Contribution of *IL-18* to inflammation and immunological responses places this cytokine into a group of potential proteins involved in the pathogenesis of autoimmune diseases such as SLE. The *IL-18* levels are significantly increased in the blood plasma of SLE patients compared to healthy subjects, and this increase has been correlated to an increase in disease activity (6).

Many studies have revealed an association between the -137 G/C (rs187238) or -607 C/A (rs1946518) polymorphisms within the *IL-18* promoter and a predisposition to various autoimmune diseases, including SLE (7-10). Recently, a significant association was determined between the -1297C/T polymorphism, located in the *IL-18* promoter region, and the incidence of SLE (11).

We studied the frequency of -137 G/C, -607 C/A and -1297C/T polymorphisms in the *IL-18* promoter in patients with SLE from a sample of the Polish population. As SLE is an extremely heterogeneous disease, we also assessed the association of these polymorphisms with different SLE clinical findings in this group of patients.

Patients and methods

Patients and controls

Data for two hundred and two women fulfilling the American College of Rheumatology Classification criteria for SLE (12) were randomly collected for this study at Institute of Rheumatology in Warsaw, Poland (Table I). Controls were matched by age to the patients and consisted of three hundred and twenty-four unrelated healthy women randomly selected from blood donors and healthy volunteers. The patients and healthy subjects were Caucasian and from the same geographic area of Poland. The

Table I. Association of the -1297C/T IL-18 promoter polymorphisms with clinical manifestations in patients with SLE.

Characteristic	Genotype distribution			
	C/C (25)[a]	C/T (91)[a]	T/T (87)[a]	Odds ratio (95% CI), *p*-value[c]
Malar rash	11	42	39	
Discoid rash	7	25	23	
Phototosensitivity	12	45	42	
Oral ulcers	9	37	34	
Arthritis	11	44	42	
Serositis	4	17	14	
Renal	19	45	36	3.792 (1.446–9.947, p=0.0051)[b]
Neurologic symptoms	5	16	15	
Haematologic symptoms	6	24	22	
Immunologic symptoms	13	43	44	
ANA	25	91	87	

[a] represents the absolute number of positive patients for C/C, C/T, T/T genotypes, respectively. Genotype comparison [b] (C/C *vs*. C/T and T/T) between patients with and patients without clinical manifestations of SLE was performed by [c] Fisher's exact test.

study was approved by the Local Ethics Committee of Poznań University of Medical Sciences. Written consent for study involvement was obtained from all participants. The mean age of SLE patients at diagnosis was 34±12 years, and controls, 33±8 years.

Genotyping

DNA was obtained from peripheral leucocytes using a standard salting out procedure. Identification of the *IL-18* -1297C/T (rs360719) polymorphic variant was conducted by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR was performed employing primer pair 5' CAACAGTGATTACAAAGGAAGT 3' and 5' TAAATGGGTAGGAATAAGTGAGA 3'. The PCR- amplified fragments of *IL-18* were 474 bp in length and were then isolated and digested with the endonuclease NlaIII (CATG/) (New England Biolabs, Ipswich, USA). The *IL-18* T allele was cleaved into 295bp and 179bp, whereas the *IL-18* C allele remained uncut. DNA fragments were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining. The presence of the *IL-18* -1297C/T polymorphism was also confirmed by commercial sequencing analysis.

The *IL-18* -607 C/A (rs1946518) and -137 G/C (rs187238) polymorphic variants were obtained by PCR with the primer pair 5' CGAAATAAAGTGGCAGAGGATA 3' and 5' TCCAGGAATAGAAAGTTTTAACA 3', followed by direct sequencing of PCR-amplified fragments of 652bp in length.

Statistical analysis

The prevalence of genotypes in patients and controls was examined for deviation from Hardy-Weinberg equilibrium. The Chi^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. The Odds Ratio (OR) and 95% Confidence Intervals (95% CI) were calculated. Contribution of the *IL-18* polymorphisms to clinical manifestations of SLE and the production of autoantibodies were determined by Fisher's exact test. Power analysis was performed using the Chi-square test, available at an online internet service, http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize.

Results

Prevalence of the IL-18 *-1297 C/T, -607 C/A and -137 G/C polymorphisms in SLE patients and controls*

Genotype analysis of all studied polymorphisms did not exhibit significant divergence form Hardy-Weinberg equilibrium in any group.

The frequency of the *IL-18* -1297CC genotype was approximately 2.4-fold times higher in patients with SLE compared to the controls, and amounted to 12.3% and 5.2% in those groups, respectively (Table II). The distribution of the *IL-18* -1297CT heterozygous genotype was also higher in patients as compared to controls, and reached 44.8% and 40.4%, respectively. The Odds ratio (OR) for SLE patients with the *IL-18* -1297CC genotype was 2.536 (95% CI=1.333 – 4.826; p=0.0035, p_{corr}=0.0105) (Table II). OR for the CC and CT genotypes was 1.586 (95% CI=1.113–2.258; p=0.0104, p_{corr}=0.0312). The statistical power of this study amounted to 81% for -1297CC and 73% for 1297CT and -1297CC genotypes.

We also found a significantly higher frequency of the *IL-18* -1297C allele in patients than in controls, amounting to 34.7% and 25.5%, respectively (Table II). The OR for the C allele in patients with SLE was 1.558 (95% CI=1.189-2.041, p=0.0013, p_{corr}=0.0039) (Table II). However, there were no statistical differences between the distribution of *IL-18* -607A/C and -137G/C genotypes and alleles in SLE patients and controls (Table II).

Contribution of IL-18 -1297C/T, -607 C/A and -137 G/C genotypes to clinical symptoms of SLE and the presence of autoantibodies in patients with SLE

We observed an association between the *IL-18* -1297CC vs CT and TT genotypes with renal manifestations of SLE, OR=3.792 (1.446-9.947, p=0.0051), however this p-value did not remain statistically significant after Bonferroni correction (p_{corr}= 0.0867) (Table I). There was no correlation between the *IL-18* -607 C/A and -137 G/C polymorphisms and clinical manifestations of SLE. We also did not find significant associations between the presence of anti-Smith, anti-dsDNA, anti-snRNP, anti-Ro, anti-Scl-70 and anti-phospholipid antibodies to the *IL-18* -1297C/T, -607 C/A and -137 G/C polymorphisms. There were no significant differences in SLE disease activity index (SLEDAI) at diagnosis with respect to *IL-18* investigated genotype groups.

Discussion

An increased production of *IL-18* may contribute to the pathogenesis of auto-

Table II. Distribution of the -1297C/T, -607 C/A and -137 G/C IL18 promoter polymorphisms in SLE patients and controls.

		Genotype distribution absolute number (frequency %)			Allele absolute number (frequency %)		Odds ratio (95% CI)	*p*-value[d]
-1297C/T (rs360719)	n.	CC	CT	TT	C	T	2.536 (1.333–4.826)[a]	0.0035
	Controls 324	17 (5.2)	131 (40.4)	176 (54.3)	165 (25.5)	483 (74.5)	1.586 (1.113–2.258)[b]	0.0104
	SLE 203	25 (12.3)	91 (44.8)	87 (42.9)	141 (34.7)	265 (65.3)	1.558 (1.189–2.041)[c]	0.0013
-607 C/A (rs1946518)	n.	AA	CA	CC	A	C	1.204 (0.7136–2.032)[a]	0.4859
	Controls 324	38 (11.7)	153 (47.2)	133 (41.1)	229 (35.3)	419 (64.7)	1.049 (0.7334–1.500)[b]	0.7940
	SLE 203	28 (13.8)	94 (46.3)	81 (39.9)	150 (36.9)	256 (63.1)	1.072 (0.8282–1.388)[c]	0.5969
-137 G/C (rs187238)	n.	CC	CG	GG	C	G	1.545 (0.7622–3.132)[a]	0.2244
	Controls 324	17 (5.2)	139 (42.9)	168 (51.9)	173 (26.7)	475 (73.3)	1.131 (0.7963–1.607)[b]	0.4908
	SLE 203	16 (7.9)	88 (43.3)	99 (48.8)	120 (29.6)	286 (70.4)	1.152 (0.8748–1.517)[c]	0.3133

The odds ratio was calculated for patients [a] homozygous carying risk allele *vs*. homozygous or heterozygous, [b]homozygous or heterozygous carying risk allele *vs*. homozygous, [c] risk allele *vs*. major allele, [d] uncorrected Chi^2.

immune disorders, including type I diabetes, rheumatoid arthritis, Crohn's disease and SLE (5, 6). Studies employing murine MRL/lpr models have also demonstrated that *IL-18* plays a significant role in the development of lupus-like disorder (13).

The effect of the *IL-18* -137 G/C and -607 C/A polymorphisms on *IL-18* expression has been demonstrated in several studies (7). However, the role of various polymorphisms located in *IL-18* and its promoter in the susceptibility to SLE and occurrence of clinical manifestations is inconsistent across various studies (9-11). We did not observe an association of the *IL-18* -137 G/C and -607 C/A polymorphisms with SLE incidence and any manifestation of SLE. These differences between Asiatic populations and ours could result from genetic heterogeneity and exposure of these populations to disparate environmental factors.

However, we found a significant association of the *IL-18* -1297C gene variants with the occurrence of SLE in a sample of the Polish population. Our results were consistent with the findings of Sánchez *et al*. (2010), who reported a significant contribution of -1297C to the occurrence of SLE in Spanish, Italian and Argentinian cohorts (11). They used electrophoretic mobility shift assay and Western blot to demonstrate that the *IL18* -1297T variant contains a binding site for the inhibitory transcription factor OCT-1. In contrast the *IL-18* -1297C-risk gene variant could not bind OCT-1, leading to an increase in *IL-18* transcription (11). To date, a contribution of the *IL-18* -1297C gene variant has also been found in biopsy-proven giant cell arteritis (GCA) (14). Palomino-Morales *et al*. (2010) showed that, in patients with GCA, there is a significantly increased number of individuals with the IL18-1297CC or -1297CT genotypes as compared to individuals bearing the T/T genotype (14).

We also found that the *IL-18* -1297CC genotype may be associated with renal symptoms of SLE. Since the *IL-18* -1297C variant can be responsible for increased *IL-18* transcription, we presume that this cytokine can be abundantly produced in the kidney of SLE individuals with the CC genotype. Abundant biosynthesis of *IL-18* may increase the attraction of immune cells and augment inflammatory events in nephritic glomeruli. Tucci *et al*. (2008) demonstrated that patients with severe lupus nephritis exhibited a high accumulation of *IL-18* within glomeruli, attracting plasmacytoid dendritic cells and causing renal failure (15). The increased level of *IL-18* was also responsible for increased proteinuria and glomerulonephritis in lupus-prone MRL/lpr mice (13).

Our findings and the findings of other authors suggest that *IL-18* could be a new target in the treatment of SLE. However, to more precisely establish the role of *IL-18* promoter polymorphisms in SLE incidence or clinical manifestations of SLE, further studies of these variants in other populations are required.

Acknowledgements.
The technical assistance of Ms Monika Świerczyńska is gratefully acknowledged.

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