IL-10 SINGLE NUCLEOTIDE POLYMORPHISMS AND LUPUS-NEPHRITIS

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ABSTRACT

A pilot study was carried out to investigate the association of five single nucleotide polymorphisms of the IL-10 gene (-3575T/A, -2849G/A, -2763C/A, -1082G/A, -592C/A) with the risk of lupus-nephritis in Bulgarian female patients. We found a strong association between the -592 AA and CA genotypes (P = 0.009, OR 4.38, 95%CI 1.4-13.6) and the A allele (P = 0.037, OR 2.25, 95%CI 1-5) with the disease. Although statistically insignificant, the frequency of the IL-10 -3575TT, -2763CC, -2849GG and -1082GG genotypes was higher in the patients compared to the controls, which resulted in an increased odds ratio (OR). The haplotype analysis revealed an association between the IL-10 -3575T/-2849G/-2763C/-592A haplotype and lupus-nephritis (P = 0.04).

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Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease of unknown origin that affects several organ systems, mainly the kidneys (lupus-nephritis), the heart, the joints, the skin, the lungs and the brain. It is a relatively infrequent disease with a frequency of 38 to 100 per 100 000 and affects mainly women (80%) between 20 and 40 years of age.

The etiology of SLE is unknown but a key role in the induction and development of the disease is played by the imbalance between Th-1 and Th-2 cytokine production. The switch from a type 1 (Th-1) to a type 2 (Th-2) T-helper has been demonstrated with the increased serum levels of IL-4, IL-6 and IL-10 and the decreased levels of IL-2 and IFN-γ (14, 21). Among these cytokines IL-10 is believed to have an important function in the SLE pathogenesis and in the induction of disease flare (9, 10). IL-10 is mapped in 1q31–32, which is a susceptibility region for SLE (11) homologous to the murine SLE susceptibility region (18). IL-10 is a powerful inhibitor of the monocyte/macrophage activation and blocks the expression of the pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8 and IL-12 (6, 7). It also has a stimulating effect on the production of B-cells and auto-antibodies. The production of IL-10 is reported to be under strong genetic influence (22).

The objective of the present study was to determine whether IL-10 single nucleotide polymorphisms are risk factors for the development of lupus-nephritis in Bulgarian female patients and to define their contribution to the increased risk.

Materials and Methods

Subjects

Twenty female patients with systemic lupus erythematosus (SLE) were included in this pilot study. The mean age was 40, ranging from 15 to 78 years. The patients have been followed for a mean of 10 years at Medical University – Sofia, Department of Dermatology and Venereology and Department of Nephrology, and at the Ministry of Interior Hospital – Sofia, Department of Nephrology. All the patients with SLE met the American College of Rheumatology (ACR) criteria. All of them showed renal involvement to a different extent. Twelve of them also had arthritis or arthralgias, 19 either had malar rash or discoid rash and/or photosensitivity and/or oral ulceration. ANA positive titer was observed in fifteen patients. Forty-six anonymous non-related healthy individuals, matched for sex, age and ethnicity with the patients were included for genetic analysis. They were selected from the BioBank of the Molecular Medicine Center and National Genetic Laboratory.

Genetic analysis

The work described here was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The study was approved by the local ethics committee at the Medical University – Sofia. All participants signed informed consent and venous blood was drawn for DNA isolation.

Genomic DNA was extracted from the peripheral blood with the Chemagen DNA purification kit, using Chemagic Magnetic Separation Module I (Chemagen AG). Five SNPs from the IL-10 promoter region were selected for investigation (-3575A/T rs1800890; -2849G/A rs6703630; –2763A/C rs6693899; −1082G/A rs1800896; −592C/A rs1800872). All SNPs were determined by PCR-restriction fragment length polymorphism analysis.

The analysis of IL-10 -3575A/T polymorphism was performed as previously described by Moraes et al. (16). The analysis of IL-10 -2849G/A and –2763A/C polymorphisms was performed as previously described by Chong et al. (3). The analysis of IL-10 −1082G/A and IL-10 −592C/A polymorphisms was performed as previously described by Chin et al. (2).

Statistical analysis

Allelic and genotype frequencies were compared between the lupus cases and controls using Fisher's exact test to calculate P-values for 2 × 2 tables. Where significant, data were expressed as P-value, odds ratios (OR) with exact 95% confidence intervals (CI). Test for Hardy-Weinberg equilibrium was done by χ^2 statistics. LD and haplotype analysis was performed by Haploview (Haploview Software version 3.32, Broad Institute, Cambridge, MA, USA http://www.broad.mit.edu/mpg/haploview/) (1).

Results and Discussion

All the SNPs were found to be in Hardy-Weinberg equilibrium. The observed allele and genotype frequencies of the IL-10 polymorphisms among the female patients with lupus-nephritis and the healthy female controls are summarized in **Table 1**.

The IL-10 -2763A/C and IL-10 -2849 G/A polymorphisms appear in linkage disequilibrium (D' = 0.82). The -3575T/A polymorphism is localized in the distal region of the promoter. It has been reported that the IL-10 -3575T allele is associated with high IL-10 production (8). The polymorphism -3575 T/A occurs within the putative *Pit*-1 binding site. Given the association between the female sex hormones and SLE, and the high female to male ratio (9:1) in the disease, it is noteworthy that the expression of the isoforms of *Pit*-1 is regulated in part by estrogen, and that estrogen regulates the IL-10 expression in the peripheral blood mononuclear cells of the SLE patients (12). In our study we found a higher frequency of the -3575TT genotype among the patients (65.0%) compared to the controls (47.8%), which resulted in an increased OR (OR 2.03, 95% CI 0.68-6)

The IL-10 -2849G/A polymorphism lies in the distal region of the promoter. The IL-10 –2849AA genotype is associated with lower production of IL-10 (22). The -2849G/A polymorphism has been found to be non-polymorphic for the Hong Kong Chinese population and thus not associated with SLE (3). Our results showed that the -2849GG genotype is more frequent among the female patients with lupus-nephritis (85.2%) compared to the healthy controls (73.9%), which led to an increased OR (OR 2, 95%CI 0.5-8).

TABLE 1

Genotype and allele frequencies of IL-10 polymorphisms in lupus-nephritis female patients and healthy female controls

Genotype	SLE	Controls
	N = 20	N = 46
-3575T/A		
TT	13 (65.0%)	22 (47.8%)
TA	6 (30.0%)	23 (50.0%)
AA	1 (5.0%)	1 (2.2%)
T	80.0%	72.8%
A	20.0%	27.2%
P-value	**NS**	
-2849G/A		
GG	17 (85.2%)	34 (73.9%)
GA	3 (14.8%)	10 (21.7%)
AA	0 (0.0%)	2 (4.4%)
G	85.0%	84.8%
A	15.0%	15.2%
P-value	**NS**	
-2763C/A		
CC	13 (65.0%)	26 (56.5%)
CA	6 (30.0%)	16 (34.8%)
AA	1 (5.0%)	4 (8.7%)
C	80.0%	73.9%
A	20.0%	26.1%
P-value	**NS**	
-1082G/A		
GG	11 (55.0%)	21 (45.6%)
GA	9 (45.0%)	25 (54.3%)
AA	0 (0.0%)	0 (0.0%)
G	77.5%	72.8%
A	22.5%	27.2%
P-value	**NS**	
-592C/A		
CC	6 (30.0%)	30 (65.2%)
CA	12 (60.0%)	11 (23.9%)
AA	2 (10.0%)	5 (10.9%)
C	60.0%	77.2%
A	40.0%	22.8%
P-value	**CA + AA, P = 0.009 A, P = 0.037**	**NS**

N: number of patients; NS: not significant

The IL-10 -2763C/A polymorphism lies in the distal region of the promoter within the putative lymphocyte-specific factor and myeloid zinc finger binding sites. Although no significant association was found between the CC homozygotes and lupus-nephritis, the frequency (65.0%) was higher compared to the controls (56.5%) and was associated with an increased OR (OR 1.43, 95% CI 0.48-4.24). The IL-10 -2763C allele was previously found to be associated with SLE in African-Americans (8).

The IL-10 -1082G/A polymorphism appears within the putative *ETS*-factor binding site (13). The IL-10 -1082G allele was found to be associated with higher production of IL-10 (19). In our study we found a higher frequency of the IL-10 -1082GG genotype among the lupus-nephritis patients (55.0%) compared to the healthy controls (45.6%), leading to an increased OR (OR 1.46, 95% CI 0.5-4.18). A recent meta-analysis revealed that this polymorphism is associated with the development of SLE mainly in the Asian population and less in the European one (17).

The IL-10 -592C/A polymorphism appears within the STAT3 binding site (4). The role of the IL-10 -592C/A polymorphism in the etiology of the autoimmune diseases in the different populations has not been completely elucidated. The IL-10 -592CC genotype was found to be associated with the development of SLE in Chinese patients (3). At the same time Mok et al. (15) found association between the A allele and the development of SLE in Chinese patients. Other authors have not observed any association of this polymorphism and SLE (5, 20). Our results showed that the -592AA and CA genotypes (P = 0.009, OR 4.38, 95%CI 1.4-13.6) and the A allele (P = 0.037, OR2.25, 95%CI 1-5) are strongly associated with the development of lupus-nephritis in Bulgarian female patients.

Several studies showed functional data that related the IL-10 production levels to the IL-10 promoter haplotypes (4, 8, 19). Haplotype frequencies for the IL-10 single nucleotide polymorphisms were compared between the cases and the controls and the IL-10 -3575T/-2849G/-2763C/-592A haplotype turned out to be associated with lupus-nephritis in Bulgarian female patients (P = 0.04).

Conclusions

Our pilot study showed that the IL-10 promoter region polymorphisms appeared associated with lupus-nephritis in Bulgarian female patients. Further analysis on a larger cohort of patients is needed to obtain more statistically significant results.

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