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The Role of CD247 Polymorphisms in Bulgarian Patients with Systemic Lupus Erythematosus

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ABSTRACT Decreased expression of the TCR ζ-chain has been reported in several autoimmune and inflammatory diseases. Recent evidence suggests that this deficiency may be due to polymorphisms in the CD247 gene. A total 52 patients with systemic lupus erythematosus (SLE) and 95 healthy controls of Bulgarian ethnicity were genotyped for 837C>G, rs1052230, 844A>T, and rs1052231 using a TaqMan genotyping assay. None of the two polymorphisms appeared associated with the diseases. On the other hand, we have found that the -837GG genotype and the G allele were associated with hematological disease. The -844AA genotype and the A allele appeared associated with the hematological disease as well. The -843AA genotype and the A allele were found to be associated with antinuclear antibody (ANA) tests and immunological disease. An association was found between the -837G allele and arthritis. The AG haplotype was found to be associated with hematological disease, ANA, and immunological disease. Our preliminary data confirm the previous findings that the CD247 polymorphisms are mainly associated with the clinical outcome of the disease and less with susceptibility.

KEY WORDS: systemic lupus erythematosus, CD247 polymorphisms

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a serious prognosis, characterized by the production of autoantibodies affecting various organs and systems. Some of the confirmed genetic alterations in SLE affect T-cells, while the ability of T-lymphocytes to establish an immune response is primarily associated with the T-cell receptor (TCR) (1). The TCR is a multi-subunit complex, comprising at least eight transmembrane units. The clonotypic TCR α- and β-chains are responsible for the antigen recognition (2), while the invariant chains of the CD3 complex (δ, ε, and γ) and two ζ polypeptides couple antigen recognition to downstream signal transduction pathways. Studies of T-cells from TCR ζ-deficient mice showed that the ζ-chain is essential for the assembly and surface expression of the TCR/CD3 complex (3). Since TCR ζ is expressed by NK cells as a component of the activating receptors NK cell protein 46, NK protein 30, and CD16 (FcγRIII, low-affinity Fc receptor for IgG) we can hypothesize that changes in TCR ζ expression could have profound effects on innate as well as adaptive immune responses (4,5).

The CD247 gene is composed of eight exons separated by distances of between 0.7 and 8 kb (6,7). The coding region of the CD247 gene has been found to be non-polymorphic (8). A detailed analysis of 3'UTR showed that the 837C>G, rs1052230, 844A>T, and rs1052231 polymorphisms are associated with low TCR ζ expression (9). The two variants of CD247 3'-UTR may reduce TCR ζ mRNA stability, resulting in TCR ζ down-regulation.

The aim of the present study was to analyze the genetic variants of CD247 as risk factors for the susceptibility and clinical expression of SLE in Bulgarian patients.

PATIENTS AND METHODS

Patients and controls

Fifty-two patients with systemic lupus erythematosus were included in this study (45 women and 7 men). The mean age was 40, with a range of 15 to 78 years. The patients were followed for a mean of 10 years at the Department of Nephrology, Medical University of Sofia. All the patients with SLE met the American College of Rheumatology (ACR) criteria.

Ninety-five anonymous non-related healthy individuals, matched for sex, age, and ethnicity with the patients, were included for genetic analysis. They were selected from the Bio Bank of the Molecular Medicine Center and National Genetic Laboratory.

Genetic analysis

The study 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 (Chemagen AG).

The analysis of C247 polymorphisms 837C>G, rs1052230, 844A>T, and rs1052231 was performed using a TaqMan genotyping assay.

Statistical Analysis

Allele and genotype frequencies were compared between SLE cases and controls using Fisher's exact test to calculate *P* values for 2×2 tables. Where significant, data were expressed as *P* values and odds ratios (OR) with exact 95% confidence intervals (CI). The test for Hardy-Weinberg equilibrium was done using χ^2 statistics.

RESULTS

The demographic and clinical data are presented in Table 1. The two polymorphisms were in Hardy-Weinberg equilibrium and in LD 0.75. None of them appeared associated with the diseases (Table 2), but they appeared to be associated with its clinical expression (Table 3). We found the -837GG genotype and the G allele were associated with hematological disease (*P*=0.004, OR 13, 95% CL 1.5-109.5; *P*=0.004, OR 10.9, 95%CI 1.4-86.7). The -844AA genotype and the A allele appeared associated with hematological disease as well (*P*=0.0003, OR 21.5, 95% CI 2.6-180.7; *P*=0.0005, OR 15.3, 95%CI 1.9-119.5). The -843AA genotype and the A allele were found to be associated with antinuclear antibodies (ANA) (*P*=0.007, OR 5.8, 95% CL 1.6-21; *P*=0.02, OR 3.1, 95%CI 1.1-8.9) and immunological disease (*P*=0.03; OR 3.8, 95% CI 1.1-12.5, *P*=0.02; OR 3.1, 95% CI 1.1-8.8). An association was found between the -837G allele and arthritis (*P*=0.04; OR 3.1, 95%CI 1-9.4). The AG haplotype was found to

Table 1. Demographic and clinical data

Disease	**SLE**	
Demographic parameters	Women/men	45/7
	Age, mean ± SD years	40±12.4
Clinical parameters	Malar rash	33 (63.5%)
	Discoid rash	11 (21.2%)
	Arthritis	36 (69.2%)
	Oral ulcer	4 (7.7%)
	Photosensitivity	30 (57.7%)
	Serositis	10 (19.2%)
	Renal disease	52 (100.0%)
	Neurological disease	12 (23.1%)
	Hematological disease	18 (34.6%)
	Immunological disease	31 (59.6%)
	ANA	36 (69.2%)

SLE: systemic lupus erythematosus; SD: Standard Deviation; EMG: electromyography; ANA: antinuclear antibodies

be associated with hematological disease (*P*=0.003), ANA (*P*=0.05) and immunological disease (*P*=0.05).

Table 2. Genotype and allele frequencies of CD247 837C>G and 844A>T polymorphisms among patients with systemic lupus erythematosus (SLE) and controls

Genotype	SLE	Controls
-837 G/C		
GG	38 (73.1%)	68 (71.6%)
GC	13 (25.0%)	25 (25.3%)
CC	1 (1.9%)	2 (2.1%)
G	89 (85.6%)	161 (84.7%)
C	15 (14.4%)	29 (15.3)
-844 A/T		
AA	34 (65.4%)	60 (63.2%)
AT	17 (32.7%)	33 (34.7%)
TT	1 (1.9%)	2 (2.1%)
A	85 (81.7%)	153 (80.5%)
T	19 (18.3%)	37 (19.5%)

DISCUSSION

The expression of CD247 was studied recently and was found to be significantly lower in patients with SLE than in healthy controls (11,12). The two SNPs, rs1052230 and rs1052231, in the 3'UTR of the gene were found to affect the CD247 expression level in both patients with SLE and healthy controls. Despite that, no association was found between the individual polymorphisms and the development of SLE in European (9), Chinese (11), and multiethnic cohorts (11). Our results also confirmed the findings that the two polymorphisms do not confer any risk for the development of the disease. However, the CD3Z -844 AA+AT genotypes are found to be associated with the development of SLE in women (13). An association was found between a haplotype carrying the two low-expression variants and SLE (9). According to the same authors, the CD247 3'-UTR SNPs may be associated with the severity rather than with the disease risk markers (9). In agreement with that observation, we have found the two polymorphisms associated with the clinical expression of SLE such as arthritis, hematological disease, ANA, and immunological disease. Our results confirm earlier findings that that low-secreting CD247 alleles and genotypes are associated with hematologic disorders, oral ulcers, and anti-ds DNA antibody production (10).

CONCLUSIONS

The major limitation of the present study was the limited number of patients analyzed. Nevertheless, our preliminary data confirm the previous findings that the CD247 polymorphisms are mainly associated with the clinical outcome of the disease and less with susceptibility. Furthermore, a recent study demonstrated that CD247 expression can serve as a biomarker for assessing the effect of chemotherapeutic and biological drugs for the treatment of subjects suffering from a chronic inflammatory condition (14).

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Table 3. Comparison between the genotypes and ACR criteria for systemic lupus erythematosus (SLE)

Genotype	GG (n=38)	GC (n=13)	CC (n=1)	p=value	AA (n=34)	AT (n=17)	TT (n=1)	*P*=value
Malar rash	25 (65.8%)	7 (53.8%)	10 (100.0%)	NS*	23 (67.6%)	9 (52.9%)	1 (100.0%)	NS
Discoid rash	8 (21.1%)	3 (23.1%)	0	NS	7 (20.6%)	4 (23.5%)	0	NS
Photosensitivity	22 (57.9%)	8 (61.5%)	0	NS	20 (58.8%)	10 (58.8%)	0	NS
Oral ulcerations	3 (7.9%)	1 (7.7%)	0	NS	3 (8.8%)	1 (5.9%)	0	NS
Arthritis	29 (76.3%)	7 (53.8%)	0	p<0.05	26 (76.5%)	10 (58.8%)	0	NS
Serositis	9 (23.7%)	1 (7.7%)	0	NS	9 (26.5%)	1 (5.9%)	0	NS
Renal disease	38 (100.0%)	13 (100.0%)	1 (100.0%)	NS	34 (100.0%)	17 (100.0%)	1 (100.0%)	NS
Neurological disease	10 (26.3%)	2 (15.4%)	0	NS	10 (29.4%)	2 (12.5%)	0	NS
Hematological disease	19 (50.0%)	1 (7.7%)	0	GG/G p<0.05	19 (55.9%)	1 (5.9%)	0	*P*<0.05
Immunological disease (dsDNA, Sm, APA)	25 (65.8%)	6 (46.2%)	0	NS	24 (70.6%)	7 (41.2%)	0	*P*<0.05
ANA	29 (76.3%)	6 (46.2%)	1 (100.0%)	NS	28 (82.4%)	7 (41.2%)	1 (100.0%)	*P*<0.05

NS: Not significant; dsDNA: antibodies to double stranded DNA; SM: anti-Smith antibodies; APA: antiphospholipid antibodies; ANA: antinuclear antibodies

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