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

**Maria Hristova[1], Zornitsa Kamenarska[2], Gyulnas Dzhebir[2], Radka Kaneva[2], Anton Vinkov[3], Vanio Mitev[2], Lyubomir Dourmishev[4]**

[1]Department of Clinical Laboratory and Clinical Immunology and Department of Nephrology, Medical University-Sofia, Sofia, Bulgaria; [2]Molecular Medicine Center, Medical University-Sofia and Department of Medical Chemistry and Biochemistry, Medical University-Sofia, Sofia, Bulgaria; [3]Hôpitaux Drome Nord, Romans-sur-Isère, France; [4]Department of Dermatology and Venereology, Medical University-Sofia, Sofia, Bulgaria

**Corresponding author:**

Zornitsa Kamenarska MD, PhD

Molecular Medicine Center

Medical University-Sofia and Department

of Medical Chemistry and Biochemistry

Medical University-Sofia

Sofia

Bulgaria

*kamenarska@yahoo.com*

Received: October 24, 2016

Accepted: November 15, 2017

**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 $\chi^2$ 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).

**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

## References:

1. Sui W, Hou X, Zou G, Che W, Yang M, Zheng C, *et al.* Composition and variation analysis of the TCR β-chain CDR3 repertoire in systemic lupus erythematosus using high-throughput sequencing. Mol Immunol. 2015;67(2 Pt B):455-64.

2. Meuer SC, Cooper DA, Hodgdon JC, Hussey RE, Fitzgerald KA, Schlossman SF, *et al.* Identification of the receptor for antigen and major histocompatibility complex on human inducer T lymphocytes. Science. 1983;222:1239-42.

3. D'Oro U, Munitic I, Chacko G, Karpova T, McNally J, Ashwell JD. Regulation of constitutive TCR internalization by the ζ-chain. J Immunol. 2002;169:6269-78.

4. Lanier LL, Yu G, Phillips JH. Co-association of CD3ζ with a receptor (CD16) for IgG Fc on human natural killer cells. Nature. 1989;342:803-5.

5. Moretta A, Bottino C, Vitale M, Pende D, Cantoni C, Mingari MC, *et al.* Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annu Rev Immunol. 2001;19:197-223.

6. Weissman AM, Hou D, Orloff DG, Modi WS, Seuanez H, O'Brien SJ, *et al.* Molecular cloning of the ζ chain of the T cell antigen receptor. Science. 1998;239: 1018-21.

7. Jensen JP, Hou D, Ramsburg M, Taylor A, Dean M, Weissman AM. Organization of the human T cell receptor ζη gene and its genetic linkage to the Fcγ RII-Fcγ RIII gene cluster. J Immunol. 1992;148:2563-71.

8. Wu J, Edberg JC, Gibson AW, Tsao B, Kimberly RP. Single nucleotide polymorphisms of T cell receptor ζ chain in patients with systemic lupus erythematosus. Arthritis Rheum. 1999;42:2601-5.

9. Gorman CL, Russell AI, Zhang Z, Cunninghame Graham D, Cope AP, Vyse TJ. Polymorphisms in the CD3Z gene influence TCR zeta expression in systemic lupus erythematosus patients and healthy controls. J Immunol. 2008;180:1060-70.

10. Li R, Yang W, Zhang J, Hirankarn N, Pan HF, Mok CC, *et al.* Association of CD247 with systemic lupus erythematosus in Asian populations. Lupus. 2012;21:75-83.

11. Martins M, Williams AH, Comeau M, Marion M, Ziegler JT, Freedman BI. Genetic association of CD247 (CD3ζ) with SLE in a large-scale multiethnic study. Genes Immun. 2015;16:142-50.

12. Takeuchi T, Suzuki K. CD247 variants and single-nucleotide polymorphisms observed in systemic lupus erythematosus patients. Rheumatology (Oxford). 2013;52:1551-5.

13. Warchoł T, Piotrowski P, Lianeri M, Cieslak D, Wudarski M, Hrycaj P, *et al.* The CD3Z 844 T>A polymorphism within the 3'-UTR of CD3Z confers increased risk of incidence of systemic lupus erythematosus. Tissue Antigens. 2009;74:68-72.

14. Baniyash M, Kanterman J, Sade-Feldman M, Ish-Shalom E. Cd247 as a biomarker for assessing the effect of chemotherapeutic and biological drugs. WO2013050998 A1, 11 April 2013.