ASSOCIATION OF -308 G/A TNFA POLYMORPHISM WITH SYSTEMIC LUPUS ERYTHEMATOSUS: A PRELIMINARY STUDY

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ABSTRACT

PURPOSE: The aim of this study was to investigate whether -308 G/A TNFA polymorphism (rs1800629) enables development of SLE in Bulgarian population. METHODS: Genotyping for rs1800629 polymorphism was performed by restriction fragment length polymorphisms-PCR assay. A total of 86 SLE patients were compared with 83 healthy subjects. RESULTS: There was an increase of the TNFA -308 A allele (21.5% vs 9%) and decrease of the TNFA -308 G allele in cases versus controls (78.5% vs 91%; p=0.001). We found a lower frequency of GG genotype (57% vs 81.9%) and a higher frequency of heterozygous AG genotype (43% vs 18%) in SLE patients compared to healthy individuals (p<0.001). Logistic regression analysis revealed that the presence of TNFA -308 A allele in the genotype was associated with 3.4 times higher risk of developing SLE (OR=3.423; p<0.001). Moreover, we observed that the carriers of the GG-genotype are protected from development of neuropsychiatric lupus (OR=0.224; p=0.048) and simultaneously have an elevated risk for hematological abnormalities (OR=2.5; p=0.086).

CONCLUSIONS: Our results shown that TNFA rs1800629 gene polymorphism is associated with susceptibility and appearance of certain clinical manifestations of this autoimmune disease. The importance of TNFA in SLE development could be useful for establishing current management strategies with biological agents.

Key words: cytokine, promoter polymorphism, SLE

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disorder with both genetic and environmental factors contributing to its development. Genetic analysis of susceptibility to SLE suggests a polygenic inheritance with the largest contribution from the major histocompatibility complex (MHC) (1). The prominent role played by tumor necrosis factor (TNF)-α in inflammation and its relevance to SLE as well as the location of the TNF-α gene on chromosome 6 within the class III region of the MHC has led to great interest in the possibility that variants of the gene might be involved into disease susceptibility (2). Individual variations in TNF production in healthy controls have been observed, with high and low producer phenotypes present in population, indicating a substantial genetic contribution to regulation of TNF synthesis (3, 4). These findings suggest that polymorphism in the TNFA regulatory region might influence its production. A number of single nucleotide polymorphisms (SNPs) within the promoter of the TNF-α gene have been identified. Among these common polymorphisms in the promoter, a G-to-A transition at position -308 (rs1800629) has been most widely analyzed. Some investigations have suggested that such allelic variations could have functional significance, but the results obtained have been inconsistent (5). Several studies have examined the potential contribution of TNFA -308G/A SNP to SLE susceptibility (6-8). However, studies on the
relationship of TNFA rs1800629 polymorphism with SLE are in certain. In this regard, the aim of this study was to investigate the role of TNFA rs1800629 polymorphism as risk factor for development of SLE and expression of certain clinical manifestations in Bulgarian population.

**PATIENTS AND METHODS**

**Patients**

A total of 86 patients with SLE attending the laboratory of Clinical Immunology, Medical Center “St. Ivan Rilski” Stara Zagora were included in this cross-sectional study as were compared with 83 healthy subjects. All participants completed questionnaire about their medical histories and rheumatologists performed a clinical evaluation on each participant. The group of SLE patients consisted of 77 (89.5%) women and 9 (10.5%) men from 16 to 71 years old with a mean (±SD) age of 41.6 ± 13.6 years. The mean (±SD) disease duration was 7.4 ± 8.0 years (range 0.4 – 42). The patients fulfilled at least four of the 1982 American College of Rheumatology (ACR) criteria for SLE (9). Clinical characteristics of SLE patients are summarized in **Table 1**. The sex and age distribution of the healthy controls (HC) was: 58 (69.9%) women and 25 (30.1%) men from 19 to 74 years old with a mean (±SD) age of 39.0 ± 10.8 years. The study was approved by the institutional ethics committee and all subjects signed an informed consent.

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuropsychiatric</td>
<td>12 (14.3%)</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>17 (20.2%)</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>32 (37.2%)</td>
</tr>
<tr>
<td>Musculo-articular</td>
<td>78 (92.9%)</td>
</tr>
<tr>
<td>Serositis</td>
<td>38 (45.2%)</td>
</tr>
<tr>
<td>Muco-cutaneous</td>
<td>71 (84.5%)</td>
</tr>
<tr>
<td>Hematological</td>
<td>62 (73.8%)</td>
</tr>
<tr>
<td>DNA positivity</td>
<td>35 (41.7%)</td>
</tr>
<tr>
<td>Antiphospholipid syndrome</td>
<td>9 (10.7%)</td>
</tr>
</tbody>
</table>

**DNA extraction**

Genomic DNA was extracted from peripheral whole blood using the NucleoSpin Blood L Purification kit (Macherey-Nagel, Duren, Germany) and stored at -80°C until use. The concentration and purity of DNA extracts was determined by DNA/RNA spectrophotometer Gene Quant 1300 at A260 and A280. The ratio of absorptions at 260nm vs 280nm was used to assess the purity of DNA samples.

**Genotyping for -308 G/A polymorphism in TNFA gene.**

Genotyping for the -308G/A polymorphism in the TNFA gene (rs1800629) was performed by restriction fragment length polymorphisms (RFLP) analysis of PCR fragment amplified using the modified forward primer 5’- AGG CAA TAG GTT TTG AGG GCC AT 3’ and the reverse primer 5’- TTG GGG ACA CAC AAG CAT CAA GG 3’ to create a restriction site for the NcoI enzyme. PCR amplification was performed in a GeneAmp PCR System 9700 (Applied Biosystems). The thermocycling conditions were as follows: 95°C for 2 minutes; 95°C for 45 seconds, 65°C for 45 seconds, and 72°C for 45 seconds for 35 cycles, and then 72°C for 5 minutes. Restriction enzyme of NcoI (Thermo Scientific) digestion with the PCR product was carried out overnight 37°C and then analyzed on a 3% agarose gel. DNA products were visualized by ethidium bromide staining and analysed by phototyping. In each PCR run, heterozygous control template was used to ensure accuracy. Sizes of PCR fragments were 150bp for -308 A allele and 128, 22bps for -308 G allele.

**Statistical analysis.**

All data for this study were analyzed using SPSS V.16.0. The differences in genotype distribution and allele frequency among cases and healthy
individuals were analyzed using the $\chi^2$ test. StatPages.net website (http://statpages.org/index.html) was used to estimate odds ratios (OR), expressed with their 95% confidence intervals (95% CI) for disease susceptibility and clinical presentation in relation to TNFA -308G/A polymorphism. The goodness of fit to Hardy-Weinberg equilibrium was performed using a $\chi^2$ test. In all cases, two-tailed p-values less than 0.05 were considered as significant.

RESULTS

Association of TNFA -308A/G polymorphism with susceptibility to SLE

The genotype distribution and allele frequencies of -308G/A in gene promoter of TNFA among cases and controls are presented in Table 2. The genotype distribution of TNFA -308G/A polymorphism was in agreement with Hardy-Weinberg equilibrium among controls ($\chi^2=1.322$; p=0.516), but not for cases ($\chi^2=6.509$; p=0.039). We found significant differences in the genotype ($\chi^2=12.344$; df=2; p<0.001) and allele ($\chi^2=10.1$; df=2; p=0.001) frequencies of TNFA -308G/A polymorphism between SLE patients and controls. There was an increase of the TNFA -308 A allele (21.5% vs 9%) and decrease of the TNFA -308 G allele in cases versus controls (78.5% vs 91%; p=0.001). We found a lower frequency of GG genotype (57% vs 81.9%) and higher frequency of heterozygous AG genotype (43% vs 18%) in SLE patients compared to healthy subjects (p<0.001). Logistic regression analysis revealed that the presence of TNFA -308 A allele in the genotype was associated with an elevated risk of developing SLE which is estimated at 3.4 (OR=3.423; 95% CI: 1.606–7.367; p=0.000).

Table 2. Distribution of allele and genotype frequencies of TNFA -308 G/A polymorphism in study patients and controls.

<table>
<thead>
<tr>
<th>rs1800629</th>
<th>AA</th>
<th>AG</th>
<th>AA/AG</th>
<th>GG</th>
<th>A</th>
<th>G</th>
</tr>
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<tbody>
<tr>
<td>TNF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC (n=83)</td>
<td>-</td>
<td>15 (18.1%)</td>
<td>15 (18.1%)</td>
<td>68 (81.9%)</td>
<td>15 (9.0%)</td>
<td>151 (91.0%)</td>
</tr>
<tr>
<td>SLE (n=86)</td>
<td>-</td>
<td>37 (43.0%)</td>
<td>37 (43.0%)</td>
<td>49 (57.0%)</td>
<td>$\chi^2=6.359$</td>
<td>37 (21.5%)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>-</td>
<td>3.423</td>
<td>3.423</td>
<td>1</td>
<td>2.759</td>
<td>1</td>
</tr>
<tr>
<td>P</td>
<td>-</td>
<td>0.000</td>
<td>0.000</td>
<td>ref.</td>
<td>(1.392–5.528)</td>
<td>ref.</td>
</tr>
</tbody>
</table>

Role of TNFA -308G/A polymorphism in clinical manifestation of SLE

The genotype distribution of SNP in -308 locus of TNFA among study patients with regard to certain clinical manifestations of SLE was investigated. An association was revealed for neuropsychiatric involvement (p=0.048) as well as for appearance of hematological abnormalities (p=0.086) under dominant model (AA + AG vs GG) (Table 3). We observed that the carriers of the GG-genotype are protected from development of neuropsychiatric lupus (OR=0.224; p=0.048) and simultaneously have a high risk for hematological manifestations (OR=2.5; p=0.086). No association of TNFA – 308G/A polymorphism with other clinical manifestations in SLE was detected (data not shown).

DISCUSSION

TNF-α is a key cytokine in the regulation of inflammation and apoptosis, two processes involved in the pathogenesis of SLE. This cytokine stimulates the production of other pro-inflammatory cytokines, enhances neutrophil activation and expression of adhesion molecules and acts as a costimulator for T-cell activation and antibody production. These effects, along with its potent immunomodulator activities (10), could support the involvement of TNF-α in the pathogenesis of SLE (11).
Polymorphism present at position −308 has been associated with different levels of cytokine production. The less common TNF2 allele (−308A) has been related to higher TNF-α transcription rate than the TNF1 allele (−308G) after in vitro activation of lymphocytes with different stimuli (12). In vivo studies on mRNA levels confirmed this association (13). TNF2 is part of the extended haplotype HLA-A1-B8-DR3-DQ2 (14), associated with high TNF-α production (15) and with predisposition to several autoimmune diseases. Nevertheless, carriage of TNF2 allele alone leads to an increase in TNF-α production that could modify cytokine homeostasis in favor of the development of pathogenic situations.

Until now, several studies have examined the association of TNFA SNP at locus -308 and susceptibility to SLE. However, the association of TNFA SNPs with SLE is still not consistent. In the current study, we established over-representation of TNFA -308 A allele in Bulgarian patients with SLE. Our results suppose that the presence of TNFA -308 minor allele A in the genotype could be a risk factor to the SLE susceptibility. It is important to emphasize that our data confirm a previous reports from other authors showing a higher frequency of TNFA -308 A in Caucasian SLE patients (8).

TNF-α genotypes associated with high cytokine production have been linked to SLE susceptibility in different populations (6, 16, 17). Thus, an increased risk of developing SLE, independent of the HLA-DR genotype, has been reported for carriers of TNF2 allele in Caucasians (2, 6, 16, 18), African American (19), Chinese (20), Colombian (16, 17) and Mexican (21) populations, while no relation was found in a few studies analyzing mestizo Mexican (22), African Americans (7) or Asian (23) cohorts. In fact, the allele-based comparisons of 21 studies (8), after stratification by ethnicity, detected a significant association of the −308A allele in the European-derived groups, but not in Asian-derived or African-derived populations.

More recently performed meta-analysis summarizes results on the association of TNFA promoter −308A/G polymorphism with SLE susceptibility in Asian populations (24). A total of 12 studies (1017 cases and 1086 controls) were included in this meta-analysis (Chinese, Japanese, and Thai). A significant association of A allele and increased SLE risk was found (OR = 1.44, 95%CI = 1.04–2.01, P = 0.03). The meta-analysis demonstrates the association between TNFA −308A/G polymorphism and SLE in Asian populations, especially in Chinese population.

In addition to studies of the incidence of TNFA -308G/A polymorphism in SLE, some researchers have examined the possible associations of the allele variants with clinical signs and symptoms of the disease. However, there were no definitive data on the association of TNFα polymorphisms and specific clinical manifestations, probably due to the heterogeneity of the disease. The most analyses did not find relevant relationships with clinical parameters of the disease, although it has been reported some association (20, 25). We found that carriage of TNFA -308A allele is a protective factor for appearance of neuropsychiatric involvement and simultaneously a risk factor for hematological manifestations, thus having a modifying effect on the clinical presentation of the disease.

CONCLUSION

Our results clearly prove that TNFA rs1800629 gene polymorphism is associated not only with susceptibility, but also with appearance of certain/clinical manifestation of this autoimmune

Table 3. Association of TNFA -308G/A polymorphism with clinical manifestations of SLE

<table>
<thead>
<tr>
<th></th>
<th>GG n (%)</th>
<th>AA+AG n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neuropsychiatric manifestations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n=72)</td>
<td>34 (47.2)</td>
<td>38 (52.8)</td>
</tr>
<tr>
<td>Positive (n=12)</td>
<td>2 (16.7)</td>
<td>10 (83.8)</td>
</tr>
<tr>
<td><em>OR=0.224; 95%CI=0.031+1.21; p=0.048</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hematological manifestations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n=22)</td>
<td>20 (37.7)</td>
<td>4 (80.0)</td>
</tr>
<tr>
<td>Positive (n=62)</td>
<td>33 (62.3)</td>
<td>1 (20.0)</td>
</tr>
<tr>
<td><em>OR=2.5; 95%CI=0.779+8.318; p=0.086</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Odds ratios (ORs) under dominant model (AA+AG) only are shown.
disease. The importance of TNFA in SLE development could be useful for establishing current management strategies with biological agents.

REFERENCES


