Trakia Journal of Sciences, Vol. 12, Suppl. 1, pp 175-180, 2014

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

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

M. Ivanova[1], G. Vasilev[1], S. Stanilova[2], I. Manolova[3*]

[1]Clinic of Rheumatology, University Hospital, Medical University, Sofia, Bulgaria
[2]Department of Molecular Biology, Immunology and Medical Genetics, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
[3]Department of Health Care, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

ABSTRACT
PURPUSE: 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). Inter 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

***Correspondence to**: *Irena Manolova, MD, PhD, Department of Health Care, Medical Faculty, Trakia University, Armeiska Str 11, Stara Zagora 6003, Bulgaria, e-mail: imanolova@mf.uni-sz.bg*

relationship of *TNFA* rs1800629 polymorphism with SLE are incertain. 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 1. *Clinical data of study patients with SLE (n=86)*

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

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: 95ºC for 2 minutes; 95ºC for 45 seconds, 65ºC for 45 seconds, and 72ºC for 45 seconds for 35 cycles, and then 72ºC for 5 minutes. Restriction enzyme of *Nco*1 (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 photoyping. 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 web site (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 χ^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.*

rs1800629 ***TNF***	**AA**	**AG**	**AA/AG**	**GG**		**A**	**G**
HC (n=83)	-	15 (18.1%)	15 (18.1%)	68 (81.9%)		15 (9.0%)	151 (91.0%)
SLE (n=86)	-	37 (43.0%)	37 (43.0%)	49 (57.0%)	***χ^2=6.359 p=0.000***	37 (21.5%)	135 (78.5%)
OR (95% CI) *P*	-	**3.423** (1.606÷ 7.367) ***0.000***	**3.423** (1.606÷ 7.367) ***0.000***	1 ref.		**2.759** (1.392÷5.528) ***0.001***	1 *ref.*

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).

DISCUSION

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).

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

	GG n (%)	AA+AG n (%)
Neuropsychiatric manifestations		
Negative (n=72)	34 (47.2)	38 (52.8)
Positive (n=12)	2 (16.7)	10 (83.8)
*OR=0.224: 95%CI=0.031÷1.21; p=0.048		
Hematological manifestations		
Negative (n=22)	20 (37.7)	4 (80.0)
Positive (n=62)	33 (62.3)	1 (20.0)
*OR=2.5: 95%CI=0.779÷8.318; p=0.086		

*Odds ratios (ORs) under dominant model (AA+AG) only are shown

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 TNF*α* 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

disease. The importance of *TNFA* in SLE development could be useful for establishing current management strategies with biological agents.

REFERENCES

1. Harley, J.B., Moser, K.L., Gaffney, P.M., Behrens, T.W. The genetics of human systemic lupus erythematosus. *Curr Opin Immunol*, 10: 690–696, 1998.
2. Rood, M.J., van Krugten, M.V., Zanelli, E., van der Linden, M.W., Keijsers, V., Schreuder, G.M., Verduyn, W., Westendorp, R.G., de Vries, R.R., Breedveld, F.C., Verweij, C..L, Huizinga, T.W. TNF-308A and HLA-DR3 alleles contribute independently to susceptibility to systemic lupus erythematosus. *Arthritis Rheum*, 43:129–134, 2000.
3. Wilson, A.G., Di Giovine, F.S., Blakemor,e A.I..F, Duf,f G.W. Single base polymorphism in the human Tumour Necrosis Factor alpha (TNFα) gene detectable by NcoI restriction of PCR product. *Human Molecular Genetics*, 1:353, 1992.
4. Louis E., Franchimont D., Piron A., Gevaert Y., Schaaf-Lafontaine N., Roland S., Mahieu P., Malaise M., De Groote D., Louis R., Belaiche J. Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans.*Clin Exp Immunol,* 113:401-406, 1998
5. Allen RD. Polymorphism of the human TNF-alpha promoter--random variation or functional diversity? *Mol Immunol*, 36:1017-1027,1999.
6. van der Linden, M.W., van der Slik, A.R., Zanelli, E. et al: Six microsatellite markers on the short arm of chromosome 6 in relation to HLA-DR3 and TNF-308A in systemic lupus erythematosus. *Genes Immun,* 2:373–380, 2001.
7. Parks, C.G., Pandey, J.P., Dooley, M.A., Treadwell, E.L., St Clair, E.W., Gilkeson, G.S., Feghali-Bostwick, C.A., Cooper, G.S. Genetic polymorphisms in tumor necrosis factor (TNF)-alpha and TNF-beta in a population-based study of systemic lupus erythematosus: associations and interaction with the interleukin-1alpha-889 C/T polymorphism. *Hum Immunol*, 65:622–631, 2004.
8. Lee, Y.H., Harley, J.B., Nath, S.K. Meta-analysis of TNF-α promoter -308 A/G polymorphism and SLE susceptibility. *European Journal of Human Genetics*, 14: 364–371, 2006.
9. Tan, E.M., Cohen, A.S., Fries, J.F., Masi, A.T., McShane, D.J., Rothfield, N.F., Schaller, J.G., Talal, N., Winchester, R.J. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*, 25:1271–1277, 1982.
10. Hehlgans, T., Pfeffer, K. The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. *Immunology*, 115:1–20, 2005.
11. Aringer, M., Smolen, J.S. Complex cytokine effects in a complex autoimmune disease: tumor necrosis factor in systemic lupus erythematosus. *Arthritis Research and Therapy*, 5:172–177, 2003
12. Kroeger, K.M., Stee,r J.H., Joyce, D.A., Abraham, L.J. Effects of stimulus and cell type on the expression of the -308 tumour necrosis factor promoter polymorphism. *Cytokine*, 12:110–119, 2000.
13. Suárez, A., Castro, P., Alonso, R., Mozo, L., Gutiérrez, C. Interindividual variations in constitutive interleukin-10 messenger RNA and protein levels and their association with genetic polymorphisms. *Transplantation*, 75:711–717, 2003.
14. Price, P., Witt, C., Allcock, R., Sayer D, Garlepp M, Kok CC, French M, Mallal S, Christiansen F. The genetic basis for the association of the 8.1 ancestral haplotype (A1, B8, DR3) with multiple immunopathological diseases. *Immunological Reviews*, 167:257–274, 1999.
15. Abraham LJ, French MAH, Dawkins RL. Polymorphic MHC ancestral haplotypes affect the activity of tumour necrosis factor-alpha. *Clinical and Experimental Immunology*, 92:14–18, 1993.
16. Guarnizo-Zuccardi, P., Lopez, Y., M. Giraldo, Garcia, N., Rodrigue, L., Ramirez, L., Uribe, O., Garcia, L., Vasquez, G. Cytokine gene polymorphisms in Colombian patients with systemic lupus erythematosus. *Tissue Antigens*, 70: 376– 382, 2007.

17. Correa, P. A., Gomez, L. M. and Anaya, J. M. Polymorphism of TNF-alpha in autoimmunity and tuberculosis. *Biomedica*, 24:43–51, 2004.
18. Schotte, H., Willeke, P., Tidow, N., Domschke, W., Assmann, G., Gaubit,z M., Schlüter, B. Extended haplotype analysis reveals an association of TNF polymorphisms with susceptibility to systemic lupus erythematosus beyond HLA-DR3. *Scandinavian Journal of Rheumatology*, 34:114–121, 2005.
19. Sullivan, K. E., Wooten, C., Schmeckpeper, B. J., Goldman, D., Petri, M. A. A promoter polymorphism of tumor necrosis factor *α* associated with systemic lupus erythematosus in African-Americans. *Arthritis and Rheumatism*, 40:2207–2211, 1997.
20. Azizah, M. R., Kuak, S. H., Ainol, S. S., Rahim, M. N., Normaznah, Y., Norella, K. Association of the tumor necrosis factor alpha gene polymorphism with susceptibility and clinical-immunological findings of systemic lupus erythematosus. *Asian Pacific Journal of Allergy and Immunology*, 22:159–163, 2004.
21. Jimenez-Morales, S., Velazquez-Cruz, R., Ramırez-Bello, J., Bonilla-González, E., Romero-Hidalgo, S., Escamilla-Guerrero, G., Cuevas, F,, Espinosa-Rosales, F., Martínez-Aguilar, N.E., Gómez-Vera, .J, Baca, V., Orozco, L. Tumor necrosis factor-*α* is a common genetic risk factor for asthma, juvenile rheumatoid arthritis, and systemic lupus erythematosus in a Mexican pediatric population. *Human Immunology*, 70:251–256, 2009.
22. Zuniga, J., Vargas-Alarcon, G., Hernandez-Pacheco, G., Portal-Celhay, C., Yamamoto-Furusho, J. K., Granados, J. Tumor necrosis factor-α promoter polymorphisms in Mexican patients with systemic lupus erythematosus (SLE). *Genes and Immunity*, 2:363–366, 2001.
23. Hirankarn, N., Wongpiyabovorn, J., Hanvivatvong, O., Netsawang, J., Akkasilpa, S., Wongchinsri, J., Hanvivadhanakul, P., Korkit, W., Avihingsanon, Y. The synergistic effect of FC gamma receptor IIa and interleukin-10 genes on the risk to develop systemic lupus erythematosus in Thai population. *Tissue Antigens*, 68:399–406, 2006.
24. Zou. Y.F., Feng, X.L., Tao, J.H., Su, H., Pan, F.M., Liao, F.F., Fan, Y., Ye, D.Q. Meta-analysis of TNF-α promoter –308A/G polymorphism and SLE susceptibility in Asian populations. *Rheumatol International*, 31:1055-1064, 2011.
25. Lin, Y.-J., Chen, R.-H., Wan, L., Sheu, J..J-C., Huang, C.-M., Lin, C.-W., Chen, S.-Y., Lai, C.-H., Lan, Y.-C., Hsueh, K.-C., Tsai, C.-H., Lin, T.-H., Huang, Y.-M., Chao, K., Chen, D.-Y. and Tsa, F.-J. Association of TNF- *α* gene polymorphisms with systemic lupus erythematosus in Taiwanese patients. *Lupus*, 18:974–979, 2009.