

Tumor Necrosis Factor Promoter Polymorphisms in Patients with Rheumatoid Arthritis in Taiwan

JENG-HSIEN YEN, CHUNG-JEN CHEN, WEN-CHUN TSAI, CHIA-HUI LIN, TSAN-TENG OU, CHENG-CHIN WU, and HONG-WEN LIU

ABSTRACT. *Objective.* To investigate the association of tumor necrosis factor (TNF) promoter polymorphisms with rheumatoid arthritis (RA) in Taiwan.

Methods. TNF promoter polymorphisms at positions -238, -244, -308, -376, -857, and -863 were determined in 97 patients with RA and 97 healthy controls using the PCR-RFLP method.

Results. The phenotypic frequency of TNF-308A was significantly lower in patients with RA than in healthy controls. This finding can only be found in HLA-DR4 negative patients, not in DR4 positive RA patients and controls. The TNF promoter polymorphisms at positions -238, -244, -308, -376, -857, and -863 were not related to the clinical manifestations of RA patients.

Conclusion. TNF-308A itself or a neighboring gene may be a protective factor for the development of RA in the HLA-DR4 negative population in Taiwan. TNF promoter polymorphisms were not associated with the clinical manifestations of patients with RA in Taiwan. (J Rheumatol 2001;28:1788-92)

Key Indexing Terms:

TUMOR NECROSIS FACTOR POLYMORPHISM RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a heterogeneous disease of unknown etiology with genetic predisposition. The HLA class II antigens¹, especially HLA-DR, have been thought to be related to the pathogenesis of RA. The strongest association in Taiwanese patients is with the DR4 alleles, especially DRB1*0405². However, the shared epitope hypothesis accounted for only half of the patients in our previous study. Therefore, the genetic association of non-HLA alleles with RA should be investigated.

Tumor necrosis factor- α (TNF- α) is a proinflammatory cytokine. It is associated with the inflammatory reaction in patients with RA³. Several papers have shown that TNF- α may be related to the pathogenesis of RA^{4,7}. Transgenic mice with a modified human TNF- α gene can develop an erosive arthritis mimicking RA⁸. Treatment with anti-TNF- α monoclonal antibody or TNFR2 improved the clinical manifestations of patients with RA^{9,10}.

Several polymorphisms have been noted in TNF promoter^{11,12}. Some reports have shown that production of

TNF- α could be influenced by TNF promoter polymorphisms, TNF microsatellites, and HLA-DR genes¹³⁻¹⁵. Therefore, the TNF promoter polymorphisms may play a role in the pathogenesis of RA. There were many reports about the associations of TNF promoter polymorphisms with malaria infection, primary biliary cirrhosis, and some autoimmune diseases including RA, juvenile RA, systemic lupus erythematosus, psoriatic arthritis, and ankylosing spondylitis^{12,16-22}. However, the associations between TNF promoter polymorphisms and these diseases are still controversial²³⁻²⁸.

A report about the association of this gene with RA is unavailable in Taiwan. We investigated the role of TNF promoter polymorphisms in the pathogenesis of RA in Taiwan.

MATERIALS AND METHODS

To investigate the role of TNF promoter polymorphisms in the pathogenesis of RA, 97 patients with RA and 97 healthy controls were enrolled in this study. The 1987 revised American College of Rheumatology criteria for classification of RA were used for the diagnosis of RA. All patients and controls are Taiwanese.

The polymorphisms at TNF-238, -244, -308, -376, -857, and -863 were determined. TNF-238, -244, and -376 polymorphisms were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. A set of primers with mismatched nucleotides (underlined) were used. The sequences of primers were 5'-CCTCAAG-GACTCAGCTTTCTG-3' and 5'-ACACTCCCCATCCTCCCCAGATC-3'. The amplification was accomplished by initial denaturation at 96°C for 1 min and 5 cycles at 96°C for 45 s, at 58°C for 1 min, and 72°C for 1 min, then 30 cycles at 96°C for 45 s, at 53°C for 1 min, and 72°C for 1 min; and final extension at 72°C for 6 min (Perkin-Elmer 9600).

The restriction enzymes used for determining TNF-238, -244, and -376 polymorphisms were Bgl II, BsaI, and Tsp509 I, respectively. The sequences with TNF-238G, TNF-244G, and TNF-376A can be digested by

From the Division of Allergy, Immunology, and Rheumatology, Department of Internal Medicine, Kaohsiung Medical University Hospital, and Department of Internal Medicine, Kaohsiung Municipal Hsiao-Kang Hospital, Taiwan, ROC.

J-H. Yen, MD, PhD, MSc, Associate Professor; C-J. Chen, MD, MSc, Associate Professor; W-C. Tsai, MD, Assistant Professor; C-H. Lin, Technician, Division of Allergy, Immunology, and Rheumatology, Kaohsiung Medical University; T-T. Ou, MD, Attending Physician, Department of Internal Medicine, Kaohsiung Municipal Hsiao-Kang Hospital; C-C. Wu, MD, Clinical Fellow; H-W. Liu, MD, Professor, Department of Internal Medicine, Kaohsiung Medical University.

Address reprint requests to Dr. J-H. Yen, Division of Allergy, Immunology, and Rheumatology, Department of Internal Medicine, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung City 807, Taiwan.

Submitted August 29, 2000; revision accepted February 12, 2001.

Bgl II, BsaI, and Tsp 509 I, respectively. Because none of the PCR products from patients and controls could be digested by Tsp 509 I, a fragment of hypoxanthine-guanine phosphoribosyl-transferase gene was amplified as the positive control for Tsp 509 I during enzyme digestion. There is a restriction site within this PCR product. The sequences of primers were 5'-GAAGTGTCTCAGCAAATGG-3' and 5'-GCTCAGTCCATAACAAGC-3'. The amplification was accomplished by an initial denaturation at 94°C for 5 min, and 5 cycles at 94°C for 1 min, at 55°C for 1 min, and 72°C for 1 min, followed by 30 cycles at 94°C for 1 min, at 50°C for 1 min, and at 72°C for 1 min; and a final 3 min 72°C elongation phase.

The TNF-308, TNF-857, and TNF-863 polymorphisms were also determined by PCR-RFLP method^{29,30}. The sequences of primer sets for amplifying TNF-308, TNF-857, and TNF-863 were 5'-AGCAATAGGTTTGAGGGCCAT-3', and 5'-TCCTCC CTGCTCCGATCCG-3', 5'-GGCTCTGAGGAATGGGTTAC-3' and 5'-CCTCTACATGGCCCTGTCTAC-3', 5'-GGCTCTGAGGAATGGGTTAC-3', and 5'-CTACATGGCCCTGTCTTCGTTACG-3', respectively. The underlined nucleotides are mismatched. NcoI and TaiI were used as the restriction enzyme. The sequences with TNF-308G, TNF-857C, and TNF-863A can be digested by NcoI, TaiI, and TaiI, respectively. The restriction sites of these polymorphisms are shown in Table 1.

Extraarticular involvement of RA was considered when at least one of the following clinical manifestations was present during the disease course: (1) subcutaneous rheumatoid nodule; (2) cutaneous vasculitis; (3) eosinophilia; (4) lymphadenopathy; (5) pulmonary diseases (pleurisy, interstitial fibrosis, nodular lung or pulmonary hypertension); (6) cardiac disease (pericarditis or conduction defect confirmed by echocardiography and electrocardiography); (7) noncompressive neuropathy; (8) Raynaud's phenomenon; or (9) sicca syndrome (confirmed by positive Schirmer's test and positive lip biopsy). Radiographs of hands, wrists, and feet were taken in patients. Evaluation of bone erosion was performed by a radiologist and a rheumatologist.

The chi-square test with Yates' correction or Fisher's exact test was used for statistical analysis. Odds ratio (OR) was calculated by the methods of Woolf³¹ and by a modification of the method of Haldane³².

RESULTS

Table 2 shows the frequencies of TNF promoter polymorphisms in patients with RA and healthy controls. The allele and phenotypic frequencies of TNF-308A were significantly lower in patients than in controls. There were no significant differences in the frequencies of TNF-308G, TNF-238, TNF-857, and TNF-863 polymorphisms between patients and controls.

Table 3 shows the phenotypic frequencies of TNF promoter polymorphisms in HLA-DR4 positive and DR4 negative RA patients and controls. The frequency of TNF-308A was significantly lower in DR4 negative patients than

in DR4 negative controls, but not in DR4 positive patients or controls.

Table 4 gives the comparisons of prevalences of TNF promoter polymorphisms in different populations. The prevalences of these polymorphisms were variable in different populations. The prevalences of TNF-238A, -244A, -308A, and -857T in Taiwanese were compatible with those in American whites, while the prevalence of TNF-863A was slightly higher in Taiwanese. The prevalence of TNF-308A was markedly lower in Japanese than in other populations, and the prevalence of TNF-376A in Taiwanese was comparable with that in Dutch individuals.

DISCUSSION

TNF- α is an important mediator in the inflammatory response of RA. The production of TNF- α may be associated with TNF promoter polymorphisms, TNF microsatellites, and HLA-DR genes. Several polymorphisms have been found in TNF gene at positions -1031, -863, -857, -575, -376, -308, -244, -238, and +70. Positions -238 and -244 are located within a sequence similar to the so-called Y box, a regulative motif typical of the promoter region of MHC class II genes, or to a repressor site³³. The TNF repressor site has been localized to a 25 bp region between base pair -254 and -230 in the promoter³⁴. The variations of sequence in this area may affect TNF promoter activity. However, Drouet's study showed that the TNF Y box bound an abundant nuclear factor but had no detectable activity³⁵.

The associations between TNF- α production and TNF promoter polymorphisms are controversial. Wilson's study showed that TNF-308A is a much stronger transcriptional activator than TNF-308G in the human B cell line, and the TNF-308 polymorphisms have direct effects on TNF gene regulation³⁶. However, Uglialoro's study showed that the TNF-308 polymorphisms did not affect the TNF gene expression in activated lymphocytes¹². In Abdallah's study, the TNF-308A was related to the lower plasma TNF- α level, but not significantly so³⁷. As for position -244, the association between TNF- α production and polymorphisms at this nucleotide is still unavailable. The polymorphisms at TNF-376 will affect the expression of TNF- α . An adenine substitution (TNF-376A) had a 35% increase in basal expression compared to wild-type TNF-376G³⁸.

Table 1. Restriction sites of TNF promoter polymorphisms by PCR-RFLP methods.

Restriction Enzyme	TNF-238		TNF-244		TNF-308		TNF-376		TNF-857		TNF-863	
	G	A	G	A	G	A	G	A	C	T	C	A
Bgl II	+	-										
BsaI			+	-								
Nco I					+	-						
Tsp 509 I							-	+				
Tai I									+	-	-	+

+: positive cutting site; -: no cutting site.

Table 2. Frequencies of TNF promoter polymorphisms in patients with RA and healthy controls.

	RA, n = 97 (%)	Controls, n = 97 (%)	OR	p
Genotype frequencies				
TNF-238 G/G	94 (96.9)	96 (99.0)		
G/A	3 (3.1)	1 (1.0)		
A/A	0 (0)	0 (0)		
TNF-308 G/G	94 (96.9)	72 (74.2)		
G/A	3 (3.1)	23 (23.7)		
A/A	0 (0)	2 (2.1)		
TNF-857 C/C	65 (67.0)	72 (74.2)		
C/T	27 (27.8)	24 (24.8)		
T/T	5 (5.2)	1 (1.0)		
TNF-863 C/C	82 (84.5)	73 (75.3)		
C/A	12 (12.4)	21 (21.6)		
A/A	3 (3.1)	3 (3.1)		
Phenotype frequencies				
TNF-238 G	97 (100.0)	97 (100.0)	1.0	NS
A	3 (3.1)	1 (1.0)	3.1	NS
TNF-308 G	97 (100.0)	95 (97.9)	5.1	NS
A	3 (3.1)	25 (25.7)	0.1	< 0.0001
TNF-857 C	92 (94.8)	96 (98.9)	0.2	NS
T	32 (32.9)	25 (25.8)	1.4	NS
TNF-863 C	94 (96.9)	94 (96.9)	1.0	NS
A	15 (15.5)	24 (24.7)	0.6	NS
Allele frequencies				
TNF-238 A	3 (1.5)	1 (0.5)	3.0	NS
TNF-308 A	3 (1.5)	27 (13.9)	0.1	< 0.0001
TNF-857 T	37 (19.1)	26 (13.4)	1.5	NS
TNF-863 A	18 (9.3)	27 (13.9)	0.6	NS

OR: odds ratio.

Table 3. Phenotypic frequencies of TNF promoter polymorphisms in HLA-DR4 positive and DR4 negative patients with RA and controls.

	DR4 (+)				DR4 (-)			
	RA, n = 46 (%)	Controls, n = 18 (%)	OR	p	RA, n = 51 (%)	Controls, n = 79 (%)	OR	p
TNF-308								
G	46 (100.0)	18 (100.0)	2.51	NS	51 (100.0)	77 (97.5)	3.32	NS
A	2 (4.3)	2 (11.1)	0.36	NS	1 (1.9)	23 (29.1)	0.05	< 0.0001

OR: odds ratio.

Table 4. Comparison of prevalences of TNF promoter polymorphisms in different populations (%).

	Ugialoro	Brinkman	Seki	Present study
Population	American white	Dutch	Japanese	Taiwanese
TNF -238A	0	9.5	4	1
-244A	0	ND	ND	0
-308A	20.8	43.1	3.3	25.8
-376A	ND	2.2	ND	0
-857T	20.8	ND	32.3	25.8
-863A	16.7	ND	26.3	24.7

ND: not determined.

Associations of other polymorphic sites with TNF- α expression have also been reported. The polymorphisms at TNF-575 and +70 were not related to TNF- α production. However, the correlations of TNF-863 and -857 polymorphisms to TNF- α expression are still controversial. Increase of, no effect on, and decrease of TNF- α expression have been reported in TNF-863 polymorphisms^{12,30,39}, and no effect on or increase of TNF- α transcription has been reported in TNF-857 polymorphisms^{12,39}.

Because the polymorphisms at TNF promoter may be associated with different expressions of TNF- α , these polymorphisms may be related to some inflammatory diseases.

The correlations of TNF promoter polymorphisms with RA are still controversial. Danis, *et al* showed that the genotypic frequency of TNF-308A in patients with RA was found to be 3 times that of healthy controls¹⁸. However, the associations of TNF-376, -308, -238, and +70 alleles with susceptibility to RA could not be found by Brinkman, *et al*⁴⁰. Vinasco, *et al* also showed that there were not significant associations of TNF-308 and -238 polymorphisms with susceptibility to RA⁴¹.

In this study, the polymorphisms at positions -244 and -376 could not be found. The allele and phenotypic frequencies of TNF-308A were significantly lower in patients than in controls. After stratification with HLA-DR4, the phenotypic frequency of TNF-308A, was significantly lower in DR4 negative patients with RA than in DR4 negative controls. The same finding cannot be found in DR4 positive patients and controls. Although a linkage disequilibrium between TNF-308A and HLA-DR3 was noted in Caucasians, it was not found in Taiwanese. Nor was linkage disequilibrium found between TNF-308A and other HLA-DR in Taiwanese. There was no significant difference in the phenotypic frequencies of HLA-DR alleles between DR4 negative RA patients and DR4 negative controls (data not shown). Therefore, the negative association between TNF-308A and RA was not secondary to HLA-DR. TNF-308A itself or a neighboring gene may be a protective factor for the development of RA in HLA-DR4 negative populations. This finding is different from previous reports. The reason for this discrepancy needs to be investigated.

Seki, *et al* showed that the frequency of TNF-857T allele was significantly higher in RA patients versus controls²⁷. However, it was secondary to the linkage disequilibrium of TNF-857T with HLA-DRB1*0405. A similar finding could not be found in this study. In this study, TNF-238, -857, and -863 polymorphisms were not related to the development of RA. Seki, *et al* also showed that TNF-238 and -863 were not susceptible genes to RA.

Vinasco, *et al* showed that TNF-238 and TNF-308 polymorphisms were related to mean age at disease onset and nodular disease, respectively⁴¹. Brinkman, *et al* also showed that TNF-238GA genotype was associated with decreased radiologically detectable progression. They also found that the polymorphisms at positions +70, -308, and -376 were not related to the severity of RA⁴⁰. The same findings could not be confirmed in our study. We showed that TNF-238, -308, -857, and -863 polymorphisms were not associated with extraarticular involvement, bone erosion, seropositivity for rheumatoid factor, or age at disease onset. This finding is compatible with Lacki, *et al*⁴². They showed that TNF-308 gene polymorphism did not affect the clinical and radiological outcomes of RA.

The prevalences of TNF promoter polymorphisms in different populations were variable even within Caucasians and within Orientals, and the associations of TNF promoter

polymorphisms with RA were also different. In Taiwanese and Japanese, the prevalences of TNF-238A, -857T, and -863A were similar to those in American whites, while TNF-308A was markedly lower in Japanese than in other populations. The prevalences of TNF-238A and -308A were markedly higher in Dutch patients than in other populations. The genetic bases of different populations were different, and the associations between TNF gene and RA were also different.

In summary, TNF-308A is negatively associated with susceptibility to RA in the HLA-DR4 negative population in Taiwan. TNF-308A itself or a neighboring gene may be a protective factor for the development of RA in this population. TNF promoter polymorphisms were not related to the clinical manifestations of patients with RA in Taiwan.

ACKNOWLEDGMENT

We thank Nancy Nai-Chia Liu for her assistance in laboratory work.

REFERENCES

1. McCusker CT, Singal DP. Molecular relationships between the class II HLA antigens and susceptibility to rheumatoid arthritis. *J Rheumatol* 1988;15:1050-3.
2. Yen JH, Chen JR, Tsai WJ, Tsai JJ, Liu HW. HLA-DRB1 genotyping in patients with rheumatoid arthritis in Taiwan. *J Rheumatol* 1995;22:1450-4.
3. Husby G, Williams RC Jr. Synovial localization of tumor necrosis factor in patients with rheumatoid arthritis. *J Autoimmun* 1988;1:363-71.
4. Saklatvala J. Tumor necrosis factor alpha stimulates resorption and inhibits synthesis of proteoglycan in cartilage. *Nature* 1986;322:547-9.
5. Brennan FM, Maini RN, Feldmann M. Role of pro-inflammatory cytokines in rheumatoid arthritis. *Springer Semin Immunopathol* 1998;20:133-47.
6. Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. *Annu Rev Immunol* 1996;14:397-440.
7. Beutler B, Bazzoni F. TNF, apoptosis and autoimmunity: a common thread? *Blood Cells Mol Dis* 1998;24:216-30.
8. Keffer J, Probert L, Cazlaris H, et al. Transgenic mice expression human tumor necrosis factor: a predictive genetic model of arthritis. *EMBO J* 1991;10:4025-31.
9. Moreland LW, Schiff MH, Baumgartner SW, et al. Etanercept therapy in rheumatoid arthritis. A randomized, controlled trial. *Ann Intern Med* 1999;130:478-86.
10. Elliott MJ, Maini RN, Feldmann M, et al. Repeated therapy with monoclonal antibody to tumor necrosis factor alpha (cA2) in patients with rheumatoid arthritis. *Lancet* 1994;344:1125-7.
11. Matsushita M, Tsuchiya N, Nakayama T, et al. Allele typing of human TNFA 5'-flanking region using polymerase chain reaction-preferential homoduplex formation assay (PCR-PHFA): linkage disequilibrium with HLA class I and class II genes in Japanese. *Tissue Antigens* 1999;54:478-84.
12. Uglialoro AM, Turbay D, Pesavento PA, et al. Identification of three new single nucleotide polymorphisms in the human tumor necrosis factor-alpha gene promoter. *Tissue Antigens* 1998;52:359-67.
13. Hamann A, Mantzoros C, Vidal-Puig A, Flier JS. Genetic variability in the TNFA promoter is not associated with type II diabetes mellitus (NIDDM). *Biochem Biophys Res Commun* 1995;211:833-9.

14. Pociot F, Briant L, Jongeneel CV, et al. Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF α and TNF β by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. *Eur J Immunol* 1993;23:224-31.
15. Turner DM, Grant SCD, Lamb WR, et al. A genetic marker of high TNF α production in heart transplant recipients. *Transplantation* 1995;60:1113-7.
16. McGuire W, Knight JC, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D. Severe malarial anemia and cerebral malaria are associated with different tumor necrosis factor promoter alleles. *J Infect Dis* 1999;179:287-90.
17. Jones DE, Watt FE, Grove J, et al. Tumor necrosis factor-alpha promoter polymorphisms in primary biliary cirrhosis. *J Hepatol* 1999;30:232-6.
18. Danis VA, Millington M, Hyland V, Lawford R, Huang Q, Grennan D. Increased frequency of the uncommon allele of a tumor necrosis factor alpha gene polymorphism in rheumatoid arthritis and systemic lupus erythematosus. *Dis Markers* 1995;12:127-33.
19. Alfonso S, Colombo G, Della Bella S, Scorza R. Association between polymorphisms in the TNF region and systemic lupus erythematosus in the Italian population. *Tissue Antigens* 1996;47:551-5.
20. Hohler T, Kruger A, Schneider PM, et al. A TNF α promoter polymorphism is associated with juvenile onset psoriasis and psoriatic arthritis. *J Invest Dermatol* 1997;109:562-5.
21. McGarry F, Walker R, Sturrock R, Field M. The -308.1 polymorphism in the promoter region of the tumor necrosis factor gene is associated with ankylosing spondylitis independent of HLA-B27. *J Rheumatol* 1999;26:1110-6.
22. Hohler T, Schaper T, Schneider PM, Meyer zum Buschenfelde KH, Marker-Hermann E. Association of different tumor necrosis factor alpha promoter allele frequencies with ankylosing spondylitis in HLA-B27 positive individuals. *Arthritis Rheum* 1998;41:1489-92.
23. Rudwaleit M, Tikly M, Khamashta M, et al. Interethnic differences in the association of tumor necrosis factor promoter polymorphisms with systemic lupus erythematosus. *J Rheumatol* 1996;23:1725-8.
24. Fraile A, Mieto A, Beraun Y, Vinasco J, Mataran L, Martin J. Tumor necrosis factor gene polymorphisms in ankylosing spondylitis. *Tissue Antigens* 1998;51:386-90.
25. Kaijzel EL, Brinkman BMN, van Krugten MV, et al. Polymorphism within the tumor necrosis factor a promoter region in patients with ankylosing spondylitis. *Human Immunol* 1999;60:140-4.
26. Chen CJ, Yen JH, Tsai WC, et al. The TNF2 allele does not contribute towards susceptibility to systemic lupus erythematosus. *Immunol Letters* 1997;55:1-3.
27. Seki N, Kamizono S, Yamada A, et al. Polymorphisms in the 5'-flanking region of tumor necrosis factor-alpha gene in patients with rheumatoid arthritis. *Tissue Antigens* 1999;54:194-7.
28. Brinkman BM, Huizinga TW, Breedveld FC, Verweij CL. Allele-specific quantification of TNFA transcripts in rheumatoid arthritis. *Hum Genet* 1996;97:813-8.
29. Wilson AG, di Giovine FS, Blakemore AIF, Duff GW. Single base polymorphism in the human tumor necrosis factor alpha gene detectable by NcoI restriction of PCR product. *Hum Mol Genet* 1992;1:353.
30. Skoog T, van't Hooft FM, Kallin B, et al. A common functional polymorphism (C \rightarrow A substitution at position -863) in the promoter region of the tumor necrosis factor a (TNF α) gene associated with reduced circulating levels of TNF α . *Hum Mol Genet* 1999;8:1443-9.
31. Woolf B. On estimating the relation between blood group and disease. *Ann Hum Genet* 1955;20:251-3.
32. Haldane JBS. The estimation and significance of the logarithm of a ratio of frequencies. *Ann Hum Genet* 1955;20:309-11.
33. D'Alfonso S, Richiardi PM. A polymorphic variation in a putative regulation box of the TNFA promoter region. *Immunogenetics* 1994;39:150-4.
34. Fong CLW, Siddiqui AH, Mark DF. Identification and characterization of a novel repressor site in the human tumor necrosis factor a gene. *Nucleic Acids Res* 1994;22:1108-14.
35. Drouet C, Shakhov AN, Jongeneel CV. Enhancers and transcription factors controlling the inducibility of the tumor necrosis factor a promoter in primary macrophages. *J Immunol* 1991;147:1694-700.
36. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor a promoter on transcriptional activation. *Proc Natl Acad Sci USA* 1997;94:3195-9.
37. Abdallah AN, Cucchi-Mouillot P, Biteau N, Cassaigne A, Haras D, Iron A. Analysis of the polymorphism of the tumor necrosis factor (TNF) gene and promoter and of circulating TNF-alpha levels in heart-transplant patients suffering or not suffering from severe rejection. *Eur J Immunogenet* 1999;26:249-55.
38. Knight JC, Udalova I, Hill AVS, et al. A polymorphism that affects OCT-1 binding to the TNF promoter region is associated with severe malaria. *Nature Genetics* 1999;22:145-50.
39. Higuchi T, Seki N, Kamizono S, et al. Polymorphism of the 5'-flanking region of the human tumor necrosis factor a gene in Japanese. *Tissue Antigens* 1998;51:605-12.
40. Brinkman BMN, Huizinga TWJ, Kurban SS, et al. Tumor necrosis factor gene polymorphisms in rheumatoid arthritis: association with susceptibility to, or severity of, disease? *Br J Rheumatol* 1997;36:516-21.
41. Vinasco J, Beraun Y, Nieto A, et al. Polymorphism at the TNF loci in rheumatoid arthritis. *Tissue Antigens* 1997;49:74-8.
42. Lacki JK, Moser R, Korczowska I, Mackiewicz S, Muller W. TNF-alpha gene polymorphism does not affect the clinical and radiological outcome of rheumatoid arthritis. *Rheumatol Int* 2000;19:137-40.