Fas Promoter –670 Polymorphism Is Associated with Development of Anti-RNP Antibodies in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective***. To evaluate whether the polymorphism of Fas promoter –670 is associated with susceptibility to systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) and their clinical features.

Methods. A polymerase chain reaction of a genomic DNA-restriction fragment length polymorphism was used to determine genotypes of the Fas promoter –670 in 87 patients with SLE, 87 with RA, and 87 healthy controls. A second cohort of SLE patients (n = 85) was included. Clinical manifestations were analyzed in each patient and correlated with the genotypes.

Results. The genotype distribution of the Fas promoter –670 did not differ between patients with SLE and control subjects (AA, GA, GG genotypes 31, 54, 15% vs 30, 55, 15% controls, respectively; chi-squared = 0.03, 2 df, p = 0.99) and between RA patients and controls (AA, GA, GG genotypes 38, 44, 18% vs 30, 55, 15% controls, respectively; chi-squared = 2.30, 2 df, p = 0.32). Regarding the clinical status of lupus patients according to Fas promoter –670 genotypes, there was no significant difference in age at onset, anti-dsDNA titer, C3, C4 level, renal involvement, number of American College of Rheumatology (ACR) criteria met, SLE Disease Activity Index, SLE International Collaborating Clinics/ACR Damage Index, or autoantibody profiles. However, the frequency of anti-RNP antibody was significantly different in the AA, GA, and GG groups (71, 25, 30%; chi-squared = 13.29, 2 df, p = 0.001). To confirm this finding, the Fas promoter –670 genotype was examined in a second cohort of SLE patients (n = 85). The result in the second cohort replicated the association shown in the first. In patients with RA, there was no significant difference in clinical and laboratory findings according to the Fas promoter –670 genotypes.

Conclusion. Our data suggest that the Fas promoter –670 polymorphism is associated with development of anti-RNP antibodies in SLE. (J Rheumatol 2001;28:2008–11)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS RHEUMATOID ARTHRITIS
FAS POLYMORPHISMS

Systemic lupus erythematosus (SLE) is the prototype of human autoimmune diseases and a disorder of generalized autoimmunity with unknown etiology, characterized by multisystemic organ involvement, polyclonal B cell activation, and production of autoantibodies. One possible explanation for the pathogenesis of SLE is a failure of apoptosis of self-reactive T cells[1]. Tolerance to self-antigens can be broken and can result in autoimmune diseases such as SLE. Fas/Fas ligand interactions involve one pathogenesis by which a lymphocyte might undergo apoptosis[2]. Fas mediates apoptosis of many types of cells, such as lymphocytes, epithelial, fibroblast, and endothelial cells. It is possible that dysfunction of Fas might contribute to the pathogenesis of SLE.

Rheumatoid arthritis (RA) is a chronic inflammatory systemic autoimmune disease, characterized by synovial cell proliferation and T lymphocyte accumulation within the synovial tissue, and is associated with immune dysregulation. This may result from increased migration of activated T cells, proliferation *in situ*, or inhibition of T cell death[3]. In spite of extensive efforts, the pathogenesis of RA remains obscure. The susceptibility to RA is influenced by a major genetic component such as the human leukocyte antigen (HLA) complex, but the HLA-DR4 gene accounts for only 30–60% of RA cases[4], suggesting that there are other genetic markers associated with RA. A number of genes have been proposed, but to date little is known about genes, other than HLA, in RA.

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A recently described polymorphism within the Fas gene promoter is an A to G substitution at nucleotide position –670 in the enhancer region[5]. This polymorphism abolishes the binding site of the nuclear transcription element, gamma-activated sequence (GAS). The function of the polymorphism remains unclear, but its location, situated on a consensus sequence of the GAS, implies that it might be associated with altered Fas gene transcription. We investigated whether the polymorphism of the Fas promoter is associated with susceptibility to SLE and RA and their clinical features.

MATERIALS AND METHODS

Patients and controls. Eighty-seven Korean patients with SLE (83 female, 4 male, age 16 to 62 yrs, mean 36), 87 Korean patients with RA (73 female, 14 male, age 16–75 yrs, mean 45), and 87 ethnically matched healthy controls were enrolled in this study. The patients were recruited from the Rheumatology Clinic of Korea University Guro Hospital. A second cohort of SLE patients (82 female, 3 male, age 18 to 63 yrs, mean 37) was recruited from the Rheumatology Clinic of Korea University Anam Hospital. All patients fulfilled the classification criteria of the American College of Rheumatology (ACR) for SLE[6] or RA[7]. Eighty-seven healthy individuals with no history of an autoimmune disease were recruited as controls (68 female, 19 male, age 18–70 yrs, mean 41). SLE patient records were carefully studied and the clinical presentation, renal involvement, and autoantibody profile were recorded. Renal involvement was defined as proteinuria > 0.5 g/day or biopsy-proven lupus nephritis. We used the highest values for the anti-dsDNA titer and C3 level. The number of ACR criteria for SLE met, the SLE Disease Activity Index (SLEDAI)[8], and the Systemic Lupus International Collaborating Clinics (SLICC)/ACR Damage Index[9] were determined in each patient.

In patients with RA, clinical manifestations were determined in each patient: age at onset, functional status class according to ACR criteria[10], physician global assessment (PGA), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), and severe disease. The PGA was the physician's subjective opinion of disease severity on a visual analog scale of 0 (not severe) to 10 (most severe). ESR was determined by the Westergren method and CRP and RF were measured by nephelometry. The following variables were also evaluated: (1) history of total joint replacement (TJR) surgery, (2) history of hospitalization for RA for reasons other than TJR, (3) extraarticular manifestations such as nodules, vasculitis, eye and lung involvement, and (4) cervical spine involvement.

DNA preparation. Blood samples from all subjects were obtained for DNA extraction. Blood was collected in EDTA tubes and DNA was extracted using the method of proteinase K treatment and phenol/chloroform extraction.

Polymorphism typing of the Fas promoter. A polymerase chain reaction of a genomic DNA-restriction fragment length polymorphism using MvaI (PCR-RFLP) was used to determine genotypes of the Fas promoter –670. PCR was carried out using a forward primer 5'-CTACCTAAGAGCTATC-TACCGTTC-3' and a reverse primer 5'-GGCTGTCCATGTTGTG-GCTGC-3'. Using a Perkin-Elmer-Cetus 9600 thermal cycler, samples were subjected to 30 cycles of 30 s at 94°C for denaturing, 30 s at 58°C for annealing, and 30 s at 72°C for extension and a final extension of 4 min at 72°C. PCR products were further subjected to RFLP analysis with the enzyme Mva I and separated on a 3% agarose gel. The digested A allele yielded a fragment of 232 bp and the G allele yielded a 188 bp fragment (Figure 1).

Statistical analysis. The genotype and allele frequencies in SLE patients were compared to those in control subjects using the chi-square test. The association of age at onset, number of ACR criteria for SLE, SLEDAI, SLICC/ACR Damage Index, anti-dsDNA antibody titer and C3 level with each genotype was analyzed by the Wilcoxon or Kruskal-Wallis test. The chi-square test was also used to evaluate comparison of the frequency of renal involvement and autoantibodies in each genotype. In patients with RA, the association of clinical features with each genotype was analyzed by the chi-square, Wilcoxon, or Kruskal-Wallis test. A p value was corrected by the number of characteristics studied and a $p < 0.05$ was considered significant.

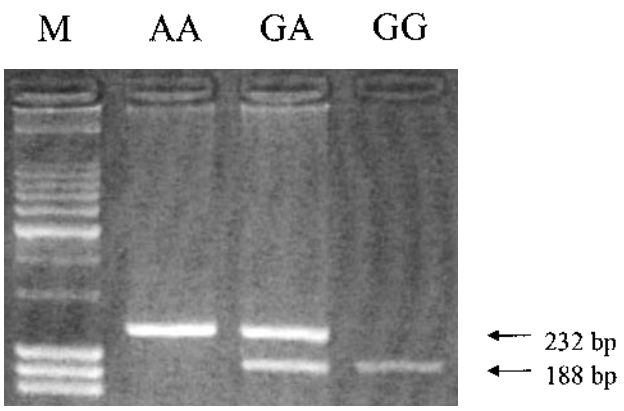

Figure 1. Fas promoter –670 genotypes by PCR-RFLP. The genotypes AA (232 bp), GA (188,232 bp), and GG (188 bp) are shown. M: DNA size marker.

RESULTS

Fas promoter –670 polymorphisms among subjects. The genotypes of the Fas promoter in patients with SLE and RA and controls did not deviate from the Hardy-Weinberg predictions. When the Fas promoter polymorphisms were compared between Korean and Caucasian healthy controls[11] with respect to the genotype and allele frequencies, there were no significant differences (Table 1). The genotype distribution of the Fas promoter did not differ between SLE patients and control subjects (AA, GA, GG genotypes 31, 54, 15% vs 30, 55, 15% controls, respectively; chi-squared = 0.03, 2 df, p = 0.99) and between RA patients and control subjects (AA, GA, GG genotypes 38, 44, 18% vs 30, 55, 15% controls, respectively; chi-squared = 2.30, 2 df, p = 0.32) (Table 2). There was no difference in the genotype or allele frequencies among SLE and RA patients and controls.

Clinical and laboratory analysis according to Fas promoter –670 genotypes. There was no significant difference in age at onset, renal involvement, number of ACR criteria met, SLEDAI, SLICC/ACR Damage Index, anti-dsDNA, or C3 level in the lupus patients grouped according to Fas promoter –670 genotypes. With respect to autoantibody profiles, such as RF, anti-Sm, anti-Ro, anti-La, anticardiolipin antibodies, and lupus anticoagulant, there was no significant difference in the lupus patients based on the Fas promoter –670 polymorphisms (Tables 3 and 4). However, the frequency of the anti-RNP antibody was significantly different among the AA, GA, and GG groups (71, 25, 30%; chi-squared = 13.29, 2 df, p = 0.001) (Tables 4 and 5). In the

Table 1. Fas promoter –670 polymorphism between Korean and Caucasian healthy control subjects.

	Korean, n = 87 (%)	Caucasian n = 86 (%)
Genotype frequencies[a]		
AA	26 (30)	20 (23)
GA	48 (55)	44 (51)
GG	13 (15)	22 (26)
Allele frequencies[b]		
G	74 (43)	84 (51)
A	100 (57)	88 (49)

[a] Chi-square test of heterogeneity, Korean vs Caucasian controls, chi-square = 3.27, 2 degrees of freedom (df); p = 0.20.
[b] Chi-square test of heterogeneity, Korean vs Caucasian controls, chi-square = 1.14, 1 df; p = 0.28.

Table 2. Genotype and allele frequencies of Fas promoter –670 polymorphism in RA, SLE patients and healthy controls.

	RA (n = 87)	SLE (n = 87)	Controls (n = 87)
Genotype frequencies[a]			
AA	33 (38)	27 (31)	26 (30)
GA	38 (44)	47 (54)	48 (55)
GG	16 (18)	13 (15)	13 (15)
Allele frequencies[b]			
G	70 (40)	73 (42)	74 (43)
A	104 (60)	101 (58)	100 (57)

[a] Chi-square test of heterogeneity, RA vs controls, p = 32; SLE vs controls, p = 99.
[b] Chi-square test of heterogeneity, RA vs controls; p = 74; SLE vs controls, p = 1.00.

second cohort of SLE patients, the frequency of the anti-RNP antibody was significantly different among the AA, GA, and GG groups [70% (19/27), 25% (11/44), 29% (4/14); chi-squared = 15.26, 2 df, p < 0.001] (Table 5). The result in the second cohort replicated the association shown in the first cohort.

In patients with RA, there was no significant difference in age at onset, functional class (≥ 3), ESR, CRP, RF titer, the frequency of admission, PGA, extraarticular and cervical spine involvement, or frequency of joint operation to the Fas promoter –670 genotypes (data not shown).

DISCUSSION

SLE and RA are autoimmune disorders of unknown etiology. The pathogenesis of the diseases remains obscure, but genetic factors are considered to be contributory. With respect to the genetics of SLE and RA, there has been the possibility that non-HLA genes may play a pathogenetic role.

Apoptosis is a physiological process that regulates normal homeostasis, and apoptosis is likely to contribute to the pathogenesis of autoimmune diseases by the impaired elimination of autoreactive T and B cells[12]. Fas is considered to play an important role in the regulation of the immune system by deleting autoreactive peripheral lymphocytes. Fas, as an apoptosis gene, is one of the candidate genes in SLE and RA.

We analyzed a new genetic marker, MvaI polymorphism on the Fas promoter gene, in patients with SLE and RA. No difference was noted in genotype or allele frequencies among SLE and RA patients and controls. Interestingly, however, the frequency of anti-RNP antibodies was significantly different in SLE patients among the AA, GA, and GG groups (71, 26, and 30%) and the result in the second cohort replicated the association of the Fas promoter –670 polymorphism with development of anti-RNP antibodies shown in the first cohort. Although in patients with RA, there was no significant difference according to the Fas polymorphism, our results suggest that the Fas promoter –670 polymorphism could play a role in RA.

To our knowledge, there have been 2 studies on the association of the Fas promoter –670 polymorphism with SLE or RA[11,13]. Huang, *et al*[11], showed a skewed distribution of MvaI genotypes in the first cohort of 103 Australian patients with RA, but this association was not confirmed in a second cohort. In SLE patients frequencies of MvaI alleles were not

Table 3. Clinical analysis of the lupus patients according to the Fas promoter –670 genotypes.

	Fas Promoter –670 Genotypes			
	AA (n = 27)	GA (n = 47)	GG (n = 13)	p
Age at onset, yrs	33.6 (2.2)	36.4 (1.8)	37.8 (3.3)	0.493
Renal disorder, % positive	37.0	25.5	30.8	0.580
No. of ACR criteria met	5.1 (1.3)	5.1 (1.4)	5.2 (1.3)	0.984
SLE disease activity index	9.9 (1.2)	11.3 (0.9)	8.3 (1.7)	0.281
SLICC/ACR Damage index	1.1 (0.2)	1.5 (0.2)	1.6 (1.7)	0.225
Anti-dsDNA titer, IU/ml	61 (25)	66 (16)	79 (16)	0.925
C3, mg/dl	66 (5)	58 (4)	70 (5)	0.153
C4, mg/dl	13 (1)	12 (1)	14 (1)	0.428

Values are the mean (SEM).

Table 4. Autoantibodies of the lupus patients according to the Fas promoter –670 genotypes.

	Fas Promoter –670 Genotypes			
	AA	GA	GG	p
Anti-Ro, n = 73 (%)	12/21 (57)	19/41 (48)	7/11 (64)	0.510
Anti-La, n = 72 (%)	3/20 (15)	7/41 (17)	2/11 (18)	0.969
Anti-RNP, n = 75 (%)	15/21 (71)	11/44 (25)	3/10 (30)	0.001
Anti-Sm, n = 74 (%)	5/22 (23)	9/42 (25)	1/10 (10)	0.681
Anti-ENA, n = 79 (%)	20/26 (77)	27/42 (64)	8/11 (72)	0.530
Anticardiolipin, n = 57 (%)	2/18 (11)	11/33 (33)	1/6 (7)	0.189
Lupus anticoagulant, n = 56 (%)	0/18 (0)	2/32 (6)	0/6 (0)	0.459
Antiphospholipid, n = 63 (%)	3/19 (11)	13/36 (36)	2/8 (25)	0.276
Rheumatoid factor, n = 47 (%)	5/12 (42)	7/39 (24)	2/6 (33)	0.216

Anti-ENA antibodies were defined as at least one positivity in anti-Ro, La, RNP, Sm antibodies.
n: tested number.

Table 5. Association between anti-RNP antibodies and Fas promoter –670 genotypes in SLE.

	Fas Promoter –670 Genotypes		
	AA	GA	GG
(A) First cohort			
Anti-RNP +	15 (71)	11 (25)	3 (30)
Anti-RNP –	6 (29)	33 (75)	7 (70)
(B) Second cohort			
Anti-RNP +	19 (70)	11 (25)	4 (29)
Anti-RNP –	8 (30)	33 (75)	10 (71)

Values given are the number of subjects (%). A. Chi-square = 13.29, 2 degrees of freedom, p = 0.001. B. Chi-square = 15.26, 2 degrees of freedom, $p < 0.001$.

different versus controls, but the AA genotype was significantly higher in SLE patients with photosensitivity or oral ulcers than in SLE patients without these features. Coakley, *et al*[13] examined the association of the Fas promoter polymorphism with RA and Felty's syndrome or large granular lymphocyte leukemia. No difference was found in the genotype or allele frequencies between the groups. They found no evidence that the polymorphism contributes to susceptibility in these diseases.

In our study, there was no difference in genotypes or allele frequencies between Caucasians[11] and Koreans, but the Fas promoter –670 polymorphism was suggested to play a role. However, the function of the Fas promoter –670 polymorphism is still unknown, and geographic or ethnic distribution of the polymorphism is also unknown. Studies are needed to clarify the biological significance of the Fas promoter polymorphism in other ethnic groups.

Our study has shown that the Fas promoter polymorphism does not confer susceptibility to SLE and RA, but Fas promoter –670 is associated with development of anti-RNP antibodies in SLE.

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