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APO-1/FAS Promoter (-670A/G) Polymorphisms and Risk of Lupus Nephritis in SLE Egyptian Female Patients

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Abstract

Background: Self-immunization in systemic lupus is driven by defective in apoptosis. Fas, is an apoptosis-promoting cell surface receptor. The present study evaluate the possible association between APO-1/FAS Promoter (-670A/G) Polymorphism and sFAS level with susceptibility to lupus nephritis in SLE patients. Design and Methods: This study was performed on 88 female patients with SLE (mean age, 39.82 ± 10.16 years). 82 patients with lupus nephritis (mean age, 42.50 ± 6.65 years). 150 age and sex-matched person served as controls. All participants were genotyped for the APO-1/FAS Promoter (-670A/G) Polymorphism, manifestations and serum sFAS were correlated with the genotypes. Results: Serum sFAS was significantly higher in patients with -670 AA genotype compared to others. (-670A/G) AA genotype frequencies were significantly higher in the lupus nephritis and SLE patients groups compared with the controls and were associated with increased risk for lupus nephritis and SLE development (odds ratio, 4.08 and 1.91 respectively). Conclusions: The APO-1/FAS Promoter (-670A/G) A allele can be used as a genetic marker for lupus nephritis susceptibility in SLE and was associated with high sFAS level.

Keywords

sFAS, -APO-1/FAS Promoter (-670A/G) Polymorphisms, Lupus Nephritis

1. Introduction

The etiology of SLE is still unknown, but many studies demonstrate association between the disease and genetic

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variations which are crucial to immunological response [1].

Lupus nephritis is a serious complication of SLE. Several factors have been proposed in the initiation and progression of LN. Two important factors that are suggested are apoptosis imbalance and overproduction of several cytokines [2].

Abnormalities in the apoptosis (programmed cell death) process could be related to development of SLE, and impairment of T or B cells [3].

Soluble FAS (sFAS) belongs to super family of death receptors and plays an important role in apoptotic signaling in different cells [4]. FAS gene is located on chromosomes 10q24.1 and consists of nine exons and eight introns [5].

Several single-nucleotide polymorphisms have been identified in the promoter region of the FAS gene, one of the substitutions of A to G at position—670 (FAS-670A/G). This functional polymorphism abolishes the binding site of nuclear transcription element and decreases the binding ability, thus diminishes the promoter activity and decreases the FAS-gene expression [6].

Therefore, the apoptosis genes, Fas and Fas ligand (FasL), could be suitable candidate genes in human SLE and lupus nephritis susceptibility.

The present study aimed to study the possible association between APO-1/FAS Promoter (-670A/G) Polymorphism and sFAS level with susceptibility lupus nephritis in SLE Egyptian female patients.

2. Statistical Analysis

Results were collected, tabulated, statistically analyzed by IBM personal computer and statistical package SPSS version 16 (SPSS Inc. Chicago, Illinois, USA). All data were expressed as mean ± standard deviation number and percent. A P-value of <0.05 was considered statistically significant.

3. Results

The results of the present study are presented in **Tables 1-3**.

Table 1 shows a significant increase of ESR, ANA titre, dsDNA titre, proteinuria percent and sfas level, while there is a significant decrease as regarding hemoglobin level and platelet count in each of SLE and lupus nephritis groups when compared to control group. Also, there is a significant increase in urea and creatinine level when compared the lupus nephritis group to the two other groups, while there is a significant increase of sfas level and proteinuria percent in lupus nephritis group when compared to SLE group, while there is no significant differences as regarding other parameters.

Table 2 shows a significant difference between lupus nephritis group and the other two groups as regarding APO-1/FAS Promoter (-670A/G) genotyping. AA genotype frequency was associated with 4.08 times higher risk of lupus nephritis than control and 1.91 times than SLE group, while AG genotype frequency was associated with 1.76 times higher risk of lupus nephritis than control. Also, A allele frequency is higher in lupus nephritis and it was associated with 4.16 times higher risk of lupus nephritis than control and 1.78 times than SLE group. As regarding SLE group, AA genotype frequency was associated with 2.014 times higher risk of SLE than control, while AG genotype frequency was associated with 1.87 times higher risk of SLE than control. Also, A allele frequency is higher in lupus nephritis and it was associated with 2.33 times higher risk of SLE than control.

Table 3 shows a significant differences among APO-1/FAS Promoter (-670A/G) polymorphisms three genotypes (AA, AG and GG) in patient groups as regarding SLEDA score and sfas level with increase level in AA genotypes than other two genotypes. Also, there is a significant increase in ANA, ds DNA, urea, creatinine, ESR and proteinuria percent and decrease in hemoglobin level and platelet count when AA and AG genotypes compared to GG genotype, while there is no significant differences regarding other parameters.

4. Discussion

SLE is an chronic inflammatory autoimmune disease involves multiple organs [7]. It is widely thought that self-immunization in systemic lupus is driven by defective clearance of dead and dying cells. In lupus patients, large numbers of apoptotic cells accumulate in various tissues including germinal centers [8].

The dysfunction of apoptosis may be a direct consequence of alterations in proteins/genes such as Fas, which

Table 1. Statistical comparison between the three studied group as regarding demographic data and laboratory findings.

Parameter	SLE	Lupus nephritis	Control	ANOVA	P value	Post hoc test
Age (years)	39.82 ± 10.16	42.50 ± 6.65	43.76 ± 7.22	0.764	>0.05	------
Disease duration (years)	7.93 ± 3.88	8.69 ± 2.67	------	0.583*	>0.05	------
HB% (gm/dl)	10.62 ± 1.79	10.15 ± 0.33	12.92 ± 0.45	84.58	<0.001	P1 > 0.05 P2 < 0.001 P3 < 0.001
WBCs ($\times10^3$/ml)	7.26 ± 2.14	7.39 ± 2.08	7.55 ± 1.27	0.298	0.743	------
Platelets ($\times10^3$/ml)	182.29 ± 45.61	164.00 ± 22.62	290.92 ± 62.07	84.65	<0.001	P1 > 0.05 P2 < 0.001 P3 < 0.001
DNA titer (IU/ml)	68.23 ± 68.14	93.75 ± 67.93	12.25 ± 2.96	47.07**	<0.001	P1 > 0.05 P2 < 0.001 P3 < 0.001
ANA titer (IU/ml)	116.07 ± 63.62	126.73 ± 71.32	53.43 ± 25.40	23.69	<0.001	P1 > 0.05 P2 < 0.001 P3 < 0.001
Urea (mg/dl)	29.77 ± 7.20	54.96 ± 5.51	25.52 ± 4.91	266.22	<0.001	P1 < 0.001 P2 > 0.05 P3 < 0.001
Creatinine (mg/dl)	0.88 ± 0.18	1.93 ± 0.19	0.87 ± 0.14	448.87	<0.001	P1 < 0.001 P2 > 0.05 P3 < 0.001
ESR (Mm/hr)	39.45 ± 18.69	39.46 ± 18.89	7.72 ± 1.56	72.68	<0.001	P1 > 0.05 P2 < 0.001 P3< 0.001
sFAS level (ng/ml)	3.84 ± 0.88	4.36 ± 0.68	0.51 ± 0.33	459.71	<0.001	P1 < 0.01 P2 < 0.001 P3< 0.001
Proteinuria %	0.0%	31(96.9%)	0.0%	115***	<0.001	------

*Mann whitney test; **Kruskal wallis test; ***Chi square; P1 between SLE and lupus nephritis; P2 between SLE and controls; P3 between lupus nephritis and controls.

Table 2. Statistical comparison among the three studied group as regarding genotyping.

Parameter	SLE No. = 38		Lupus nephritis No. = 32		Controls No. = 50		Chi square	P value	Odd's ratio	95% CI
	No.	%	No.	%	No.	%				
Genotyping AA	11	(28.9)	14	(43.8)	8	(16)			*1.91 **2.14 ***4.08	(0.71 - 5.14) (0.76 - 6.00) (1.46 - 11.43)
AG	16	(42.2)	13	(40.6)	14	(28)	16.56	0.002	0.94* **1.87	(0.36 - 2.45) (0.77 - 4.56)
GG*	11	(28.9)	5	(15.6)	28	(56)			1.76***	(0.69 - 4.49)
Alleles	No. = 56		No. = 64		No. = 100					
A	28	(50)	41	(64)	30	(30)	19.05	<0.001	*1.78 **2.33 ***4.16	(0.86 - 3.70) (1.19 - 4.59) (2.14 - 8.10)
G	28	(50)	23	(36)	70	(70)				

Table 3. Statistical comparison between the two patients groups according to genotyping as regarding demographic and laboratory data.

Parameter	AA	AG	GG	ANOVA	P value	Post hoc test
Age (years)	41.33 ± 10.64	40.56 ± 8.78	41.00 ± 7.38	0.073	0.929	======
SLEDA score	19.12 ± 8.34	9.69 ± 3.35	5.62 ± 2.22	33.67	<0.001	P1 < 0.001 P2 < 0.001 P3 < 0.05
Disease duration (years)	8.40 ± 3.82	8.09 ± 3.35	8.44 ± 3.81	0.079	0.925	=======
HB% (gm/dl)	10.96 ± 1.23	11.39 ± 1.19	11.90 ± 2.12	3.24	0.042	P1 > 0.05 P2 < 0.001 P3 < 0.001
WBCs ($\times10^3$/ml)	7.50 ± **1.71**	7.37 ± 2.15	7.40 ± 1.51	0.047	0.954	------
Platelets ($\times10^3$/ml)	186.21 ± 65.59	204.07 ± 64.77	268.71 ± 71.85	16.803	<0.001	P1 > 0.05 P2 < 0.001 P3 < 0.001
DNA titer (IU/ml)	67.36 ± 72.35	71.11 ± 66.46	20.82 ± 30.65	17.23	*< 0.001	P1 > 0.05 P2 < 0.01 P3 < 0.001
ANA titer (IU/ml)	113.04 ± 71.48	84.77 ± 54.84	84.78 ± 61.63	3.14	0.207*	======
Urea (mg/dl)	40.54 ± 14.18	37.06 ± 13.19	28.02 ± 10.97	10.45	<0.001	P1 > 0.05 P2 < 0.001 P3 < 0.001
Creatinine (mg/dl)	1.38 ± 0.56	1.22 ± 0.50	0.93 ± 0.33	9.81	<0.001	P1 > 0.05 P2 < 0.00 P3 < 0.01
ESR (Mm/hr)	34.63 ± 23.23	27.30 ± 20.34	18.64 ± 17.95	13.52*	0.001	P1 > 0.05 P2 < 0.01 P3 < 0.05
sFas level (ng/ml)	3.85 ± 2.06	2.88 ± 1.59	1.35 ± 1.18	*35.17	<0.001	P1 < 0.01 P2 < 0.00 P3 < 0.001
Proteinuria % Negative Positive	 20 (60.6) 13 (39.4)	 30 (69.8) 13 (30.2)	 40 (88.9) 5 (11.1)	8.73	0.013	-------

*kruskal wallis test.

is an apoptosis-promoting cell surface receptor. sFas is a variant of the Fas receptor lacking the transmembrane region. It has been detected in human sera and was found to inhibit apoptosis induced by Fas protein [9].

APO-1/Fas gene promoter was subdivided into basal promoter, enhancer region and silencer region. A GC-rich region is present upstream of the transcription start sites [10].

Single nucleotide polymorphism at nucleotide position -670 in the enhancer region, caused by A to G base change (-670G>A), occurs where transcription factor signal transducer and activator of transcription 1 (STAT-1) binds and thus has an effect on the level of APO-1/Fas expression. This substitution also creates MvaI restriction fragment length polymorphism [11].

The aim of this study to evaluate if there is association between APO-1/FAS Promoter (-670A/G) polymorphisms and susceptibility to SLE and lupus nephritis.

In this study there is increase percent of oral ulcer, discoid rash, arthritis, and serositis in AA genotypes. While increase perecent of photosensitivity and malar flush in AG genotypes in SLE and lupus nephritis patients.

In line with this result, Huang *et al*. reported the association of photosensitivity and oral ulcers with the -670

AA genotype. STAT-1 binding activity was found to be higher in -670A allele of the APO-1/Fas promoter as compared with the -670G allele and thus may be a candidate site for SLE susceptibility [12].

The present study showed that, Serum levels of sFas in the patients with lupus nephritis were significantly higher than patients with SLE. Also, levels of sFAS were in SLE patients were significantly higher than controls. and a positive correlation was also shown between sFas and SLEDA score.

The results of this study were in line with other studies Alecu *et al*., 2000 and Hao *et al*., 2006 that demonstrated the impressive action of sFas increase level in LN [13] [14].

In accordance with this study Cheng *et al* and. Jodo *et al* stated that sFas levels in the serum are a marker for evaluating SLE disease activity. sFas is present at a high concentration in about 60% of lupus patients. Frequency of positive serum sFas is much greater with high SLE disease activity index scores than with low scores [10] [15].

Telegina *et al*. described that the oligomeric form of sFas stimulates apoptotic cell death, and that its levels are increased in sera from lupus patients when compared to the monomeric form. The exact function of sFas in the pathogenesis of SLE remains to be established. Patients with SLE show increased levels of sFas when compared to healthy controls [16].

The present study releaved that AA genotype frequency was associated with 4.08 times higher risk of lupus nephritis than control and 1.91 times than SLE group. While, A allele frequency is higher in lupus nephritis and it was associated with 4.16 times higher risk of lupus nephritis than control and 1.78 times than SLE group.

Diecker *et al*., 2004 stated that, deranged clearance of apoptotic cell debris leads to the release of nuclear autoantigens. These autoantigens become clustered in apoptotic blebs that are restricted to sites of free radical generation in apoptotic cells for oxidative modification. These autoantigens when exposed on the surface of dying cells trigger an autoimmune response. Immune complexes of autoantigen/autoantibody bind to the basement membrane of different organs, especially the kidney, where these induce inflammation and may cause lupus nephritis, the most serious manifestation of SLE [17].

Also, in this study, As regarding SLE group, AA genotype frequency was associated with 2.014 times higher risk of SLE than control, While, A allele frequency is higher in lupus nephritis and it was associated with 2.33 times higher risk of SLE than control.

This in accordance with Xiang *et al.* who observed a negative association between the FAS-670 G allele and SLE susceptibility [18]. The study of Bita *et al.* show that the frequency of -670AA genotype was significantly higher in SLE patients than control group and the risk of SLE was 2.1-fold greater in subjects with AA genotype [19].

Also, studies by Molin *et al.* [20] and Kanemitsu *et al.* [21] demonstrated an association between APO-1/FAS Promoter (-670A/G) polymorphisms and SLE.

In contrast to present study Huang *et al.*, Araste *et al.*, Lee *et al* and Man-Manlu *et al.* reported no different allelic distributions at position -670 between patients and controls in an Iranian, Korean, Australian and Chinese population respectively [22]-[25].

It can be concluded that The APO-1/FAS Promoter (-670A/G) AA genotype and A allele can be used as a genetic marker for susceptibility of lupus nephritis in SLE patient, associated with high sFAS level and SLE susceptibility in Egyptian female population.

5. Material and Methods

This study was conducted on 320 female subjects, categorized into three groups, group I (88 SLE patients), group II (82 patients with lupus nephritis), and group III (150 subjects of healthy controls matched for age and sex). All participants were females. The mean age was 39.82 ± 10.16; 42.50 ± 6.65; and 43.76 ± 7.22 years, respectively. None of the healthy controls had a history of rheumatic disease. All patients were selected from Rheumatology and Internal Medicine Departments, Main University Hospital, Shebin El-Kom, Menofia University.

After approval of the local ethical committee and informed consent from each one, patients who were selected scheduled to undergo a sheet was taken to all patients subjected.

Inclusion criteria included patients with an established diagnosis for SLE. All patients were diagnosed according to the criteria of the American College of Rheumatology for SLE [26]. LN is defined as clinical and laboratory manifestations that meet ACR criteria, persistent proteinuria > 0.5 gm per day or greater than 3+ by

dipstick, and/or cellular casts including red blood cells [RBCs], hemoglobin, granular, tubular, or mixed [27] all patients with lupus nephritis were already diagnosed with renal biopsy in the nephrology unit. Exclusion criteria included all patients with SLE who had other connective tissue diseases or those with suspected mixed connective tissue disease.

The groups were evaluated for clinical (age, sex, constitutional manifestations, fever, malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, pleurisy), laboratory parameters [hemoglobin, white blood cell count, platelets count, blood urea, serum creatinine, erythrocyte sedimentation rate (ESR), ANA, anti-dsDNA, proteinuria] and disease activity. Disease activity was assessed according to the SLE Disease Activity Index (SLEDAI), which is a reliable and validated method for assessment of disease activity in SLE [28].

Sample collection and assay: I—Sampling: (a) Venous blood samples (10 ml) were withdrawn under complete aseptic condition by clean venipuncture and then dispensed into 4 tubes (two EDTA tubes one for complete blood picture and the other for extraction of DNA for PCR-RFLP of APO1 gene, tube with citrate for ESR measurement and the last plain tube for detection of ANA, Anti-dsDNA, soluble fas (sFas) levels and for chemical analysis of urea and creatinine. The samples in the plain tubes were allowed to clot for 30 minutes, then the serum was separated by centrifugation at 4000 rpm for 15 minutes, and the clear serum was separated and stored at −80 until time of assay.

(b) Urine samples for detection of proteinuria.

II—Analytic methods:

-Complete blood picture was measured with Pentra-80 automated blood counter (ABX-France-Rue du Caducee-Paris Euromedecine-BP-7290.34184 Montpellier-Cedex 4).

-ESR was determined with the classical Westergren method.

-Blood urea and serum creatinine were analyzed on auto-analyzer (SYNCHRON CX5) from Beckman (Beckman, instrument Inc., Scientific Instrument Division, Fullerton, CA92634-3100).

-ANA assayed with enzyme immunoassay (EIA) as purified antigens (ssDNA, poly-nucleosomes, mono-nucleosomes, histone complex, histone H1, histone H2A, histone H2B, histone H3, histone H4, PM-Scl-100,

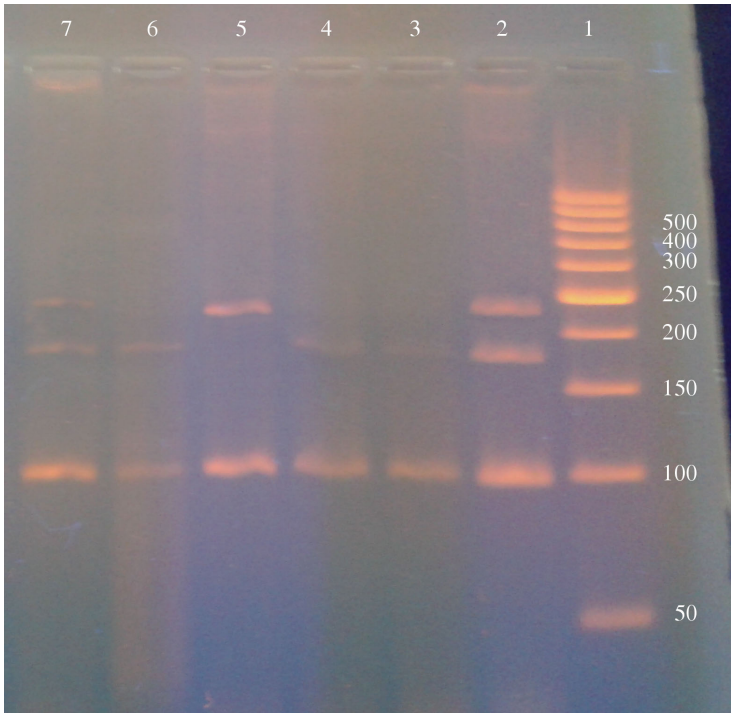

Figure 1. Shows the agrose gel electrophoresis APO-1/FAS Promoter (-670A/G) polymorphism after digestion by *MvaI* Lane 1 represent ladder (50 bp) Lanes 3, 4, 6 indicate GG genotype(188 bp and 99 bp), lanes 2.7 indicate AG genotype (233 bp, 188 bp and 99) and lane 5 indicate AA genotype (233 bp and 99 bp).

centromere B etc.) are bound to microwells. Antibodies to these antigens, if present in diluted serum bind to the respective antigens [29].

-Anti-dsDNA detected by an indirect solid phase ELISA for the quantitative measurement of IgG class autoantibodies against dsDNA in the serum [30].

-Measurement of serum sFas, using quantikine R and D sFas Immunoassay (USA), which is a solid phase ELISA. This assay employs the quantitiative sandwich enzyme immunoassay technique [31].

Proteinuria detected by dipstick test [32].

-DNA was isolated from whole blood EDTA tube samples using the QIAGEN extraction kit (Hilden, Germany), DNA eluted in buffer AE was stored at –20˚C for PCR-RFLP.

-PCR for the Genotyping for APO-1/FAS Promoter (-670A/G) Polymorphism was carried out to a total volume of 25 µl containing 10 µl of genomic DNA extract, 2.5 µl of 10× Taq polymerase buffer, 1.5 µl 2 mM $MgCl_2$ (Genecraft, Germany), 0.25 µl Taq DNA polymerase (5 units/µl) (Genecraft, Germany), 0.5 µl of dNTPS (10mM) (Stratagene, USA), 1 µl of each primer (20 µM) (Midland, Texas) & 8.25 µl of H_2O. The (-670A/G) polymorphism was detected using the following primers, forward primer. 5'-CTACCTAAGAGCTA-TCTACCGTTC-3', Reverse primer 5'-GGCTGTCCATGTTGTGGCTGC-3'. PCR amplification for this polymorphism was performed in a programmable thermal cycler Applied Biosystems 2720 (Singapore), at 94˚C for 5 minutes followed by 30 cycles at 94˚C for 30 seconds, 60˚C for 30 seconds, 72˚C for 1 minute and then one final cycle for extension at 72˚C for 7 minutes. Then the amplification products were separated by electrophoresis through 3% agarose gel stained with ethidium bromide, one band was observed (332 bp) [21].

Genotyping for APO-1/FAS Promoter (-670A/G) using restriction fragment length polymorphism.

The PCR product of the APO-1/FAS Promoter gene was digested by *MvaI* restriction enzyme (provided by Fermentas), the reaction conditions were, 6.5 µl nuclease-free water, 2.5 µl 10× buffer, 10 µl of PCR product and 1 µl (2 units) *MvaI*. The mixture was incubated for 2 hours at 37˚C then 10 µl of the products were loaded into 2% agarose gel containing ethidium bromide for electrophoresis and digestion products were resulted in 233 bp and 99 bp for A/A genotype, 233 bp, 188 bp and 99 bp for A/G genotype and 188 bp and 99 bp for G/G genotype (**Figure 1**).

Conflict of Interest

The authors declared clearly no conflict of interest.

References

[1] Lee, H.-S. and Bae, S.-C. (2010) What Can We Learn from Genetic Studies of Systemic Lupus Erythematosus? Implications of Genetic Heterogeneity among Populations in SLE. *Lupus*, **19**, 1452-1459. http://dx.doi.org/10.1177/0961203310370350

[2] Gigante, A., Gasperini, M.L., Afeltra, A., *et al*. (2011) Cytokines Expression in SLE Nephritis. *European Review for Medical and Pharmacological Sciences*, **15**, 15-24.

[3] Munoz, L.E., Van Bavel, C., Franz, S., Berden, J., Herrmann, M. and Van Der Vlag, J. (2008) Apoptosis in the Pathogenesis of Systemic Lupus Erythematosus. *Lupus*, **17**, 371-375. http://dx.doi.org/10.1177/0961203308089990

[4] Kamradt, T. and Mitchison, N.A. (2001) Tolerance and Autoimmunity. *The New England Journal of Medicine*, **344**, 655-664. http://dx.doi.org/10.1056/NEJM200103013440907

[5] Behrmann, H.W. and Krammer, P.H. (1994) Structure of the Human APO-1 Gene. *European Journal of Immunology*, **24**, 3057-3062. http://dx.doi.org/10.1002/eji.1830241221

[6] Kanemitsu, S., Ihara, K., Saifddin, A., Otsuka, T., Takeuchi, T. and Nagayama, J. (2002) A Functional Polymorphism in Fas (C D95/APO-1) Gene Promoter Associated with Systemic Lupus Erythematosus. *Journal of Rheumatology*, **29**, 1183-1188.

[7] Cantor, R.M., Yuan, J.Y., Kono, N., Napier, S., Grossman, J.M., *et al.* (2004) Systemic Lupus Erythematosus Genome Scan: Support for Linkage at 1q23, 2q33, 16q12-13, and 17q21-23 and Novel Evidence at 3p24, 10q23-24, 13q32, and 18q22-23. *Arthritis & Rheumatism*, **50**, 3203-3210. http://dx.doi.org/10.1002/art.20511

[8] Shao, W.-H. and Cohen, P.L. (2011) Disturbances of Apoptotic Cell Clearance in Systemic Lupus Erythematosus *Arthritis Research & Therapy*, **13**, 202. http://dx.doi.org/10.1186/ar3206

[9] Green, D.R. (2003) Overview: Apoptotic Signaling Pathways in the Immune System. *Immunological Reviews*, **193**, 5-9. http://dx.doi.org/10.1034/j.1600-065X.2003.00045.x

[10] Cheng, J., Zhou, T., Liu, C., Shapiro, J.P., Brauer, M.J., Kiefer, M.C., *et al*. (1994) Protection from Fas-Mediated Apoptosis by Soluble Form of the Fas Molecule. *Science*, **263**, 1759-1762. http://dx.doi.org/10.1126/science.7510905

[11] Rudert, F., Visser, E., Forbes, L., Lindridge, E., Wang, Y. and Watson, J. (1995) Identification of a Silencer, Enhancer, and Basal Promoter Region in Human CD95 (Fas/APO-1) Gene. *DNA and Cell Biology*, **14**, 931-937. http://dx.doi.org/10.1089/dna.1995.14.931

[12] Huang, Q.R., Danis, V., Lassere, M., Edmonds, J. and Manolios, N. (1999) Evaluation of a New Apo-1/Fas Promoter Polymorphism in Rheumatoid Arthritis and Systemic Lupus Erythematosus Patients. *Rheumatology*, **38**, 645-651. http://dx.doi.org/10.1093/rheumatology/38.7.645

[13] Alecu, M., Coman, G. and Alecu, S. (2000) Serological Levels of Apoptotic Bodies, sFAS and TNF in Lupus Erythematosus. *Romanian Journal of Internal Medicine*, **38**, 83-88.

[14] Hao, J.H., Ye, D.Q., Zhang, G.Q., Liu, H.H., Dai, H., *et al*. (2006) Elevated Levels of Serum Soluble Fas Are Associated with Organ and Tissue Damage in Systemic Lupus Erythematosus among Chinese. *Archives of Dermatological Research*, **297**, 329-332. http://dx.doi.org/10.1007/s00403-005-0616-2

[15] Jodo, S., Kobayashi, S., Kayagaki, N., Ogura, N., Feng, Y., Amasaki, Y., *et al*. (1997) Serum Levels of Soluble Fas/APO-1 (CD95) and Its Molecular Structure in Patients with Systemic Lupus Erythematosus (SLE) and Other Autoimmune Diseases. *Clinical & Experimental Immunology*, **107**, 89-95. http://dx.doi.org/10.1046/j.1365-2249.1997.d01-901.x

[16] Telegina, E., Reshetnyak, T., Moshnikova, A., Proussakova, O., Zhukova, A., Kuznetsova, A., *et al*. (2009) A Possible Role of Fas-Ligand-Mediated "Reserse Signalling" in Pathogenesis of Rheumatoid Arthritis and Systemic Lupus Erythematosus. *Immunology Letters*, **122**, 12-17. http://dx.doi.org/10.1016/j.imlet.2008.10.003

[17] Diecker, J.W.C., van der Vlag, J. and Berden, J.H.M. (2004) Deranged Removal of Apoptotic Cells: Its Role in the Genesis of Lupus. *Nephrology Dialysis Transplantation*, **19**, 282-285. http://dx.doi.org/10.1093/ndt/gfg485

[18] Xiang, N., Li, X.-M., Wang, G.S., Tao, J.H. and Li, X.P. (2011) Association of FAS Gene Polymorphisms with Systemic Lupus Erythematosus: A Meta-Analysis. *Molecular Biology Reports*, **40**, 407-415. http://dx.doi.org/10.1007/s11033-012-2075-0

[19] Moudi, B., Salimi, S., Mashhadi, F.F., Sandoughi, M. and Zakeri, Z. (2013) Association of FAS and FAS Ligand Genes Polymorphism and Risk of Systemic Lupus Erythematosus. *The Scientific World Journal*, **2013**, Article ID: 176741.

[20] Molin, S., Weiss, E.H., Ruzicka, T. and Messer, G. (2012) The FAS/CD95 Promoter Single-Nucleotide Polymorphism -670 A/G and Lupus Erythematosus. *Clinical and Experimental Dermatology*, **37**, 425-427. http://dx.doi.org/10.1111/j.1365-2230.2011.04296.x

[21] Kanemitsu, S., Ihara, K., Saifddin, A., Otsuka, T., Takeuchi, T. and Nagayama, J. (2002) A Functional Polymorphism in Fas (CD95/APO-1) Gene Promoter Associated with Systemic Lupus Erythematosus. *Journal of Rheumatology*, **29**, 1183-1188.

[22] Huang, Q.R., Morris, D. and Manolios, N. (1997) Identification and Characterization of Polymorphisms in the Promoter Region of Human Apo-1/Fas (CD95) Gene. *Molecular Immunology*, **34**, 577-582. http://dx.doi.org/10.1016/S0161-5890(97)00081-3

[23] Araste, J.M., Sarvestani, E.K., Aflaki, E. and Amirghofran, Z. (2010) Fas Gene Polymorphisms in Systemic Lupus Erythematosus and Serum Levels of Some Apoptosis-Related Molecules. *Immunological Investigations*, **39**, 27-38. http://dx.doi.org/10.3109/08820130903401736

[24] Lee, Y.H., Kim, Y.R., Ji, J.D., *et al*. (2011) Fas Promoter-670 Polymorphism Is Associated with Development of Anti-RNP Antibodies in Systemic Lupus Erythematosus. *Journal of Rheumatology*, **28**, 2008-2001.

[25] Man-Manlu, L.U., Ye, Q.-L., Feng, C.-C., Yang, J., Zhang, T., Li, J., Leng, R.-X., Pan, H.-F., Yuan, H. and Ye, D.-Q. (2012) Association of FAS Gene Polymorphisms with Systemic Lupus Erythematosus: A Case-Control Study and Meta-Analysis. *Experimental and Therapeutic Medicine*, **4**, 497-502.

[26] Hochberg, M.C. (1997) Updating the American College of Rheumatology Revised Criteria for the Classification of Systemic Lupus Erythematosus. *Arthritis Rheumatology*, **40**, 1725. http://dx.doi.org/10.1002/art.1780400928

[27] Dooley, M.A., Aranow, C. and Ginzler, E.M. (2004) Review of ACR Renal Criteria in Systemic Lupus Erythematosus. *Lupus*, **13**, 857-860. http://dx.doi.org/10.1191/0961203304lu2023oa

[28] Hawker, G., Gabriel, S. and Bombardier, C. (1993) A Reliability Study of SLEDAI: A Disease Activity Index for Systemic Lupus Erythematosus. *Journal of Rheumatology*, **20**, 657-660.

[29] Feltkamp, T.E. (1996) Antinuclear Antibody Determination in a Routine Laboratory. *Annals of the Rheumatic Diseases*, **55**, 723-727. http://dx.doi.org/10.1136/ard.55.10.723

[30] Egner, W. (2000) The Use of Laboratory Tests in the Diagnosis of SLE. *Journal of Clinical Pathology*, **53**, 424-432.

http://dx.doi.org/10.1136/jcp.53.6.424

[31] Nagata, S. (1994) Determination of sFas. *Advances in Immunology*, **57**, 129-135.

[32] Woolhandler, S., Pels, R.J., Bor, D.H., Himmelstein, D.U. and Lawrence, R.S. (1989) Dipstick Urine Analysis Screening of Asymptomatic Adults for Urinary Tract Disorders. *JAMA*, **262**, 1214-1219. http://dx.doi.org/10.1001/jama.1989.03430090076037