Research article

Investigation of the Relation Between FAS, FASLG Polymorphisms and Serum Fas, FasL Levels in Patients with Psoriasis

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Abstract

Background: Psoriasis is a multifactorial and inflammatory chronic skin disease indicated with T-cell-mediated keratinocyte hyper-proliferation. Demographic, epidemiological (family, twin), serological, and genetic studies have clearly demonstrated that psoriasis is a polygenic and multifactorial disease.

Aim: The objectives of the study are; to determine the prevalence of the polymorphisms of FAS (Fas cell surface receptor gene) -671 A>G (rs:1800682) and FASLG (Fas ligand gene) -844 T>C (rs:763110), to investigate the serum levels of sFas and sFasL, and also to discover any relationship between gene polymorphisms and serum levels in psoriatic patients.

Material and Methods: 50 treated and 69 untreated patients, and 140 healthy controls were included in the study. Polymorphisms were determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The serum levels were measured in randomly selected treated (39) and untreated (40) patients, also in 84 healthy controls using micro-ELISA technique.

Results: There was no statistical difference between polymorphisms in the patient and control groups. However, sFas and sFasL levels in both treated and untreated patients were higher than that of the controls.

Conclusion: The investigated FAS and FASLG polymorphisms were not found to be directly associated with the psoriasis. Elevated sFas and sFasL levels in psoriatic patients showed that these factors may possess a significant role in the pathogenesis of psoriasis.

Keywords:FAS, FASLG, Psoriasis, PCR-RFLP

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Introduction

Psoriasis is a multifactorial and inflammatory chronic skin disease indicated with T-cell-mediated keratinocyte hyper-proliferation. The disease typically appears with sharply demarcated erythematous plaques coated with silvery-white scales (1,2). Psoriasis is considered to be an autoimmune disease, the most common T-cell-dependent inflammatory disease in humans, which is caused by a combination of genetic, environmental, and immunological factors (2). The etiopathogenesis of psoriasis is multifactorial due to the interaction between genetic and enviromental factors. The genetic factors influence the formation of the disease (3,4). While psychiatric drugs, antigenic stimulation, and physical or emotional stress play an important role in triggering symptoms, most of the studies suggest that a strong genetic component causes disease susceptibility (5). It is thought that cytokines released from keratinocytes and inflammatory cells are involved in the triggering of psoriasis and the subsequent inflammatory process. It was thought that psoriasis only affects the skin. However, it has been suggested that inflammation is not limited by the definition of inflammatory arthritis in patients, and it is now claimed to be an immunological skin disease (5,6).

Apoptosis is programmed cell death that plays a role in a number of physiological events such as providing hemostasis of tissue, controlling the number of cells, and elimination of unwanted cells in organism development (7,8). It is also known to be involved in the pathogenesis of many inflammatory skin disorders. The Fas/FasL signal is among the best known mechanism for induction of apoptosis, and it is a crucial early step during the psoriasis onset promoted by activated lymphocytes (6,9). Fas-mediated apoptosis prevents the autoimmune reactions by removing the active T-cells from the medium resulting from the immunoreaction (10). In autoimmune diseases, increases in defects and Fas levels were found in the Fas pathway (11,12). The single nucleotide polymorphisms of the Fas cell surface receptor gene (*FAS*) and the Fas ligand gene (*FASLG*), particularly in the promoter region, affect the expression of these genes, and thus their serum levels (13,14). Soluble Fas (sFas) and soluble Fas ligand (sFasL) levels are important in patients with psoriasis, and there are reports that serum levels have changed due to treatment (15,16). The polymorphisms of *FAS* -671 A>G (rs: 1800682) and *FASLG* -844 T>C (rs: 763110) are found in the promoter sites of *FAS* and *FASLG* genes and have been shown in a number of studies in which these polymorphisms are associated with numerous diseases (12,13,17-19). In our study, *FAS* -671 A>G (rs: 1800682) and *FASLG* -844 T>C (rs: 763110) gene polymorphisms were preferred in order to investigate the relationship of these genes.

Specifically, we aimed to investigate whether *FAS* -671 A>G (rs: 1800682) and *FASLG* -844 T>C (rs: 763110) polymorphisms were correlated with soluble Fas (sFas) and soluble FasL (sFasL) levels in Turkish psoriasis patients.

Material and Methods

One hundred and nineteen patients with psoriasis (65 female, 54 male; average age: 41.84; SD: 16.44) who were examined in the Dermatology Clinic of Mustafa Kemal University and Cukurova University and 140 healthy control subjects (76 female, 64 male; average age: 38.7; SD: 10.2) were included in the study. The study was approved by the Ethics Committee of the Medicine Faculty of Cukurova University. Patients who were diagnosed with psoriasis according to the clinical findings and observations of the dermatologists involved in this study were included. Patients who had an inflammatory skin disease other than psoriasis and who received systemic therapy for another reason

were not included in the study. Healthy individuals with no family history of psoriasis and no relationship between them were selected for the control group. The first group of patients who received systemic treatment for psoriasis was referred to as the treatment group (50 patients). The second group of patients who received only topical treatment without systemic treatment for psoriasis was referred to as the untreated group (69 patients). All subjects included in the study received 2 ml blood samples to EDTA tubes, and DNA isolation was performed by the standard method (20). For the micro-ELISA test, 5 ml blood samples were collected from the subjects into standard biochemical tubes, and they were allowed to clot for 1 hour and centrifuged at 1.000 g. The obtained serum samples were divided into portions and were kept stored at -80 °C until used.

FAS -671 A>G and *FASLG* -844 T>C polymorphisms were analyzed with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique (21). Forward and reverse primers for *FAS* and *FASLG* were designed as follows, respectively: F:5-CTACCTA-AGAGCTATCTACCGTTC-3', R:5-GGCT-GTCCATGTGGCTGC-3'; and F:5-CAGC-TACTCGGAGGCCAAG-3, R:5-GCTCTGAG-GGGAGAGACCAT-3'. The total PCR volume was set at 25 µl for *FAS* and *FASLG* optimal amplification. All components required for PCR reaction were purchased from Fermantas company. The PCR mixture was as follows: $MgCl_2$ (50 mM): 2 µl, each primer (10 pmol): 1 µl, dNTP mixture (25 mM): 0.2 µl, Taq Polymerase (5 U/µl): 0.2 µl, PCR buffer (without $MgCl_2$): 2.5 µl and genomic DNA (50 ng/µl): 1 µl. Cycling conditions for *FAS* were set up as follows: 3 min at 94°C for the preliminary step, 40 cycles of; -1 min at 94°C for denaturation, 1 min at 62°C for annealing, and 1 min at 72°C for the extension-followed by 10 min at 72°C as a final extension using a thermal cycler (Flexigene-Techne). The

PCR product was displayed by gel electrophoresis at a length of 332 base pairs. Cycling conditions for *FASLG* were set up as follows: 2 min at 94°C for the preliminary step, 35 cycles of; -30 sec at 94°C for denaturation, 30 sec at 62°C for annealing, and 45 sec at 72°C for the extension - followed by 7 min at 72°C as a final extension using a thermal cycler (Flexigene-Techne). The PCR products were displayed with an agarose gel electrophoresis at a length of 401 base pairs.

The total volume was 25 µl for digestion by the MvaI (Fermentas) restriction endonuclease of the *FAS* PCR product. The content of the reaction mixture was as follows: MvaI: 0.5 µl, PCR product: 5 µl, and PCR buffer: 2.5 µl. It was incubated overnight at 37°C. The amplicons were run on 3% agarose gel (Sigma). The DNA fragments were monitored with a gel imaging system (Wealtec, Dolphin-View, ABD). Three different genotypes were detected as the following; AA: 233 and 99 bp, GG: 189, 99, and 44 bp, and AG: 233, 189, 99, and 44 bp fragments (Figure 1).

The total volume was set at 20 µl for digest by the BseMI (Fermentas) restriction endonuclease of the *FASLG* PCR product. The content of the reaction mixture was as follows: BseMI: 0.5 µl, PCR product: 5 µl, and PCR buffer: 1.5
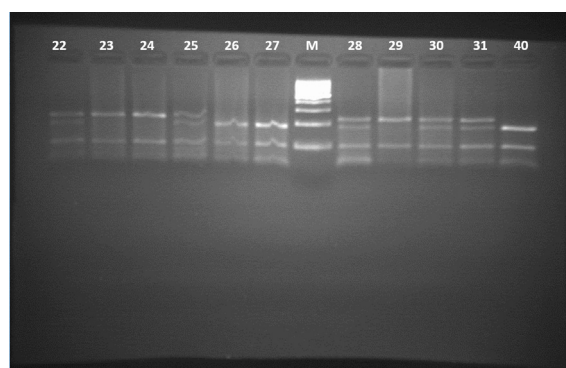
Fig. 1. Gel image of FAS -671 A>G polymorphism of patients with treated. [M: Marker (100bp). 22,25,28,30,31: AG genotype; 23,24,29: AA genotype; 26,27,40: GG

μl. It was incubated overnight at 55°C. The amplicons were run on 3% agarose gel. The DNA fragments were monitored as mentioned before. Three different genotypes were detected as the following; TT: 401 bp, CC: 233 and 168 bp, and TC: 401, 233, and 168 bp fragments (Figure 2).

The serum levels of sFas and sFasL were analyzed using micro-ELISA kits (for sFas: RayBio Human Fas ELISA; for sFas: L eBioscience Platinum ELISA) according to manufacturer instructions.

Statistical Analysis

For our data, Kolmogorov Smirnov and Shapiro Wilk tests were used as normality tests. p>0.05 was accepted as normal and parametric test was applied. Mann-Whitney U and Kruskal-Vallis tests were used to calculate the variables obtained by measurement. Mann-Whitney U was used in two group comparisons, and an Kruskal-Vallis test was used in three group comparisons. A chi-square test was conducted to compare personal variables. The level of statistical significance was p=0.05 in all tests, and a p<0.05 value was accepted as significant. The SPSS 13.0 package program was used for statistical analysis of the data.

Results

The genotype and allele frequencies were in Hardy-Weinberg equilibrium in both the pso-

Fig. 2. Gel image of FASLG -844 T>C polymorphism of control group. [M: Marker (100bp). 21,22,25,29,30,32,34,36-40: TC genotype; 23,31: CC genotype; 24,26,33,35

riasis patients and the healthy control subjects. The *FAS* -671 A>G genotype distribution and allele frequencies are listed in Table 1. When we compared *FAS* -671 AG and GG genotypes and G allele frequencies between the psoriasis and control groups, no significant difference was found (AG, p=0.521; GG, p=0.726; G, p=0.929). Serum sFas levels of patient and control groups were compared (Table 2), and we found that serum sFas levels were significantly higher in the patient group compared to the control group (p<0.001). However the average sFas level in the treated group was 200.96 pg/ml whereas it was found to be 175.07 pg/ml in the untreated group. Serum sFas levels showed no significant difference between the treated group and the untreated group (p=0.581). Serum levels of sFas, according to the three genotypes of all groups, are given in Table 3. In the control group, the sFas serum levels of AA genotypes were significantly higher than the other genotypes (p=0.001).

The *FASLG* -844 T>C genotype and allele frequencies are presented in Table 1. There was no significant difference in the *FASLG* -844 TC and CC genotype frequencies, and the C allele frequencies between the psoriasis and control groups (TC, p=0.315; CC, p=0.332; C, p=0.792). Serum sFasL levels were compared between the patient and control groups (Table 2). Serum sFasL levels in the patient group were significantly higher compared to the control group (p<0.001). However the average sFasL level in the treated group was 0.44 ng/ml whereas it was found to be 0.53 ng/ml in the untreated group. Serum sFasL levels showed no significant difference between the treated group and the untreated group (p=0.06). Serum levels of FasL were compared according to three genotypes (Table 3), and the difference in serum protein levels according to genotypes was not significant. Serum sFasL levels of all three genotypes were found to be similar in the control group as well as in the treated (p=0.753) and untreated groups (p=0.118).
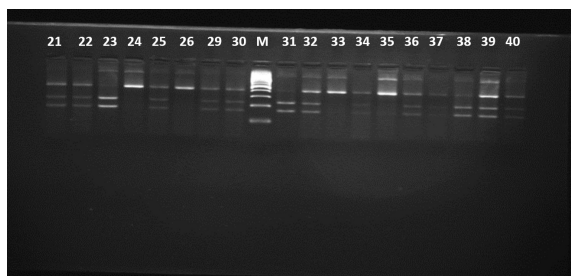
Table 1. Comparisons of genotype distribution and allele frequencies of the studied polymorphisms in patients with psoriasis and healthy controls

Genotype/Allele	Psoriasis (n=119)	Control (n=140)	X^2	OR (95% CI)	p value
FAS -671 A>G					
Genotype frequency					
AA	30 (% 25.21)	31 (% 22.14)	0.336	1,185 (0.667-2.106)	Reference
AG	71 (% 59.66)	90 (% 64.28)	0.584	0.822 (0.497-1.360)	0.521
GG	18 (%15.13)	19 (% 13.58)	1.127	1.135 (0.565-2.278)	0.726
Allele frequency					
A	131 (%55.04)	152 (% 54.28)	0.030	1.031 (0.729-1.459)	Reference
G	107 (% 44.95)	128 (% 54.72)	0.030	0.970 (0.685-1.372)	0.929
FASLG -844 T>C					
Genotype frequency					
TT	15 (% 12.60)	20 (% 14.28)	0.155	0.865 (0.422-1.776)	Reference
TC	93 (% 78.15)	101 (% 72.15)	1.235	1.281 (0.781-2.444)	0.315
CC	11 (% 9.25)	19 (% 13.57)	1.176	0.649 (0.295-1.424)	0.332
Allele frequency					
T	123 (% 51.68)	141 (% 50.36)	0.090	1.054 (0.746-1.490)	Reference
C	115 (% 48.32)	139 (% 49.64)	0.090	0.948 (0.671-1.340)	0.792

Discussion

Psoriasis is a chronic inflammatory skin disease that is seen worldwide, leading to serious disorders in the quality of life (22).

The levels of anti-apoptotic and apoptotic factors regulate the initiation of apoptosis in the epidermis, and by means of that, epidermal growth and differentiation are regulated. Completion of keratinocyte differentiation is a simple and specific form of apoptosis (23). There are various mechanisms of apoptotic pathways in keratinocyte apoptosis. One of the most important of these pathways is the extrinsic pathway induced after binding of Fas ligand (FasL) or tumor necrosis factor (TNF) to transmembrane death receptors leading to caspase-8 activation (24). In this study, we hypothesized that the signaling pathway of Fas/FasL may be impaired in the apoptotic process due to the hyperproliferation of keratinocytes. However, since poly-

morphisms of the *FAS* and *FASLG* did not significantly differ between the patient and control groups, this finding was not clear. Nonetheless, the high levels of sFas and sFasL in patients indicate that this hypothesis may still apply.

In a study of systemic lupus erythematosus (SLE) patients in order to understand the role of the Fas/FasL system in autoimmune diseases,

Table 2. Comparison of sFas and sFasL levels of patient and control groups

Groups		sFas ELISA (pg/ml)	sFasL ELISA (ng/ml)
Psoriasis (n=79)	Median	148.36	0.48
	Minimum	1.05	0.14
	Maximum	1031.06	1.06
Control (n=84)	Median	83.47	0.36
	Minimum	1.05	0.14
	Maximum	519.33	0.88
p value		p< 0.001	p< 0.001

Table 3. Distribution of sFas and sFasL levels according to genotypes in treated, untreated, and control groups

	Genotypes		Treated			Untreated			Control	
		n	Mean ± SD	Median	n	Mean ± SD	Median	n	Mean ± SD	Median
sFas (pg/ml)	AA	16	179.24 ± 86.62	179,24	4	116.57 ± 38.68	129,04	23	137.23 ± 101.84	116,77
	AG	20	216.41 ± 206.67	154,46	28	179.82 ± 119.60	143,95	43	80.91 ± 70.55	76,04
	GG	3	213.72 ± 232.90	96,62	8	187.67 ± 114.94	147,8	17	48.45 ± 60.68	83,47
	p value		0.768			0.558			0.001	
sFasL (ng/ml)	TT	6	0.47 ± 0.17	0,4	7	0.52 ± 0.17	0,46	6	0.37 ± 0.15	0,38
	TC	27	0.42 ± 0.11	0,44	29	0.52 ± 0.13	0,5	65	0.36 ± 0.12	0,36
	CC	6	0.5 ± 0.17	0,5	4	0.64 ± 0.09	0,66	13	0.39 ± 0.07	0,38
	p value		0.753			0.118			0.391	

when the levels of Fas and FasL proteins in SLE patients were found to be increased due to the severity of the disease, there was an inverse correlation between sFas levels and the stages of the disease (25). Also, in another study investigating the effects of *FAS* and *FASLG* promoter region polymorphisms on serum levels in Guillain-Barré syndrome (GBS) patients, it was reported that promoter region SNPs raised serum levels and could be associated with the disease (26). In our study, we observed that sFas and sFasL levels significantly increased in patients compared to the control group (p<0.001), which was similar to the studies mentioned above. However, no significant difference was found between treated and untreated patients [p=0.581 (Fas), p=0,06 (FasL)].

There are several previous studies investigating the role of Fas/FasL on cell apoptosis in some autoimmune diseases and various tumors (11,13,14,17,18,27,28). Furthermore, a recent study also showed that *FASLG* -844 C/T polymorphism is associated with male infertility risk, suggesting that genetic variations in the Fas/FasL system may cause spermatogenesis defects (29). In our study, there was a significant increase in sFas and sFasL levels of psoriatic patients similar to the results of various cancer studies (30).

Uncontrolled proliferation in keratinocytes may be due to impaired apoptotic mechanisms mediated by Fas/FasL, or it may be due to an immune leak. It has been reported that sFasL levels are elevated in diseases with a maculopapular rash on the skin. *FAS* and *FASLG* polymorphisms cause increased risk of certain diseases (12-14,17).

One of the surprising findings of our study is that sFas levels are high in the AA genotype individuals in the control group. Further work is needed in a wider population because the samples were taken from a small region and because our sample is of similar ethnic origin.

Also, whereas the basal expression is significantly increased in those with the -844 C allele in the *FASLG* compared to those with the -844 T allele (14), in our study, the observed distribution of sFas and sFasL levels according to genotypes in treated, untreated, and control groups, showed no significant difference between sFasL levels of the CC genotype and sFasL levels of the other genotypes.

It has been reported that the -844 CC genotype increases sFasL levels and the FasL mediated signal in lymphocytes, and, in this case, it increases the risk of developing some autoimmune diseases (2). It has also been reported that this single nucleotide polymorphism leads

to antigen-specific T-cell death, thereby increasing the cancer susceptibility of the patient (19). Unlike in the Sun et al. (2005) study, in our study, no significant correlation was found between the sFasL serum levels of the *FASLG* -844 T>C polymorphism (p>0.05). We found no significant difference between the TT, TC, and CC genotypes and the serum levels of sFasL in either the patient or the control group. However, regardless of genotypes, when the sFasL serum levels were compared between the patient group and the control group, the sFasL serum levels increased significantly in the patient group compared to the control group. We think that the expression of *FAS* and *FASLG* in psoriasis is different, but that this increase in expression is not due to *FAS* -671 A>G and *FASLG* -844 T>C polymorphisms. However, our results suggest that both *FAS* and *FASLG* may possess a crucial role in the pathogenesis of autoimmune diseases, like psoriasis. The fact that sFas and sFasL levels are close in treated and untreated patient groups, but levels of sFas and sFasL are higher in both groups of patients than in the control group suggests that further studies are needed in this regard. This significant increase in serum levels also suggests the possibility that the Fas/FasL signalling pathway is disrupted.

There are limitations in the present study as the following;

Our study is representing a relatively small number of patients. Polymorphism studies mostly reflect the genotype profile of the population better when working with greater populations in size. Additionally, while all samples were used for genotyping experiments, a limited number of samples were used for serum level measurements. In our opinion, further studies with a larger population size are needed.

Psoriasis is a still unexplained disease. For this reason, applied therapies do not offer sufficient results. Recurrence of the disease even after treatment can cause negative emotions and a lower quality of life in patients. Although it is known that the disease is triggered by environmental factors, genetic factors also clearly affect the disease. However, the lack of knowledge of genetic factors and the inability to detect a genetic defect specific to the disease prevents the development of therapy methods.

Conclusion

In this study, we investigated whether *FAS* and *FASLG* polymorphisms were effective in psoriasis. For this purpose, we analized any correlation between the serum levels of soluble Fas and FasL and the polymorphisms. We found that the polymorphisms we looked for did not affect serum levels. However, we think that we focussed on the right genes because the serum levels in the patient group were significantly higher than the ones in the control group. Therefore, other polymorphisms in these genes should also be examined. We also believe that genetic studies will have an important role to enlighten the immunopathogenesis of psoriasis. For this reason, future genetic work will be very important in understanding the genetic basis of the disease.

Abbreviations

FAS: Fas Cell Surface Receptor Gene; *FASLG*: Fas Ligand Gene; sFas: Soluble Fas; sFasL: Soluble Fas Ligand; PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

Acknowledgments

Authors' Contribution

GGD worked on plannig and all phase of study, laboratory studies; MK and RG consulted and executed the study; ACD and YGD collected the clinical specimens.

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