

Association of FAS Gene Promoter Functional Polymorphisms and the Risk of Vitiligo in Turkish Populations

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Key Words: FAS, gene, polymorphism, vitiligo, Turkish

Abstract

Background: The FAS/FASLG system shows a key role in regulating apoptosis.

Objectives: Previous findings have indicated that a significantly increased risk of vitiligo was associated with FAS-1377 AA genotype and the FAS-1377 AG genotype when compared with FAS-1377 GG genotype in Chinese population.

Material and Methods: We investigated the FAS-670, FAS-1377, FAS-844 and FAS-124 polymorphisms in 113 vitiligo patients and also compared 196 controls.

Results: We found that a significantly increased vitiligo was associated with the FAS-670 GA genotype [adjusted odds ratio (OR), 2.87; 95% confidence interval (CI), 1.37-6.03] when compared with the controls. However, there were no evident risk associated with FAS-1377, 844 and 124 genotypes.

Conclusion: This is the first report of an association between FAS-670, FAS-1377, FAS-844 and FAS-124 gene polymorphism and vitiligo in a Turkish population. The results of this study suggest that the presence of FAS-670 GA genotype can be regarded as risk factor for vitiligo, and especially for vitiligo in Turkish population.

Introduction

Vitiligo is an acquired pigmentation disorder of skin and hair, characterized by symmetrically arranged, well defined white patches, as a result of the selective destruction and disappearance of functioning melanocytes from the epidermis and follicular reservoir [1]. It is an enigmatic disease with variable clinical presentations and an unpredictable prognosis. The pathogenesis of this cosmetically disfiguring disease has still not been completely

clarified. The disappearance of melanocytes from the achromic patches appears to be multifactorial and has been so far explained as either caused by hereditary factors, auto-immunization, neurological disorders and autodestruction. Vitiligo occurs in 1-4% of the population, mostly between the ages of 10 and 30 years [2, 3]. But it is much more frequent among probands' relatives: 7.0% among all first degree relatives, 6.1% among siblings, and 23% among monozygot twins.

The genetics of vitiligo cannot be explained by simple Mendelian genetics, it is characterized by incomplete penetrance. Family clustering of cases and segregation analyses have suggested multifactorial, polygenic inheritance [4, 5].

Despite the lack of conclusive evidence, the finding of melanophagia and the absence of a prominent inflammation in vitiligo lesions favor the theory that apoptosis is responsible for the melanocyte death in vitiligo. In a recent study, Ruiz-Arguelles et al. demonstrated that residual melanocytes in biopsies from vitiligo patients expressed anti-apoptotic markers, which strongly supports that those cells where the expression of proapoptotic genes were predominant incurred rapidly in apoptosis and they are no longer found as residual melanocytes. They also showed the presence of serum antibodies to melanocytes in vitiligo patients, which penetrate into cultured melanocytes in vitro and trigger them to undergo into apoptosis [6, 7]. Apoptosis has also recently been proposed as a mechanism for intracellular redistribution and exposure and unusual cleavage fragments of autoantigens. The exposure of previous cryptic determinants on apoptotic cells could consequently cause an autoimmune response which may induce autoimmunity in vitiligo [8, 9].

Autoimmune mechanism stems from the fact that many vitiligo patients also exhibit other autoimmune disorders and some patients respond to immunosuppressive treatments. Melanocytes are particularly immunogenic by the contents of their melanosomes, generating melanogenic enzymes and structural components, including tyrosinase, MART-1, gp100, TRP-1 and TRP-2. These molecules are also prime targets of the immune response in both vitiligo and melanoma. An autoimmune response targeting epidermal melanocytes has been demonstrated in vitiligo patients which tends to be subclinical. Studies demonstrated circulating antibodies and auto reactive T cell, particularly cytotoxic T cell, infiltrations at the margins of lesions against these melanosomal antigens in affected subjects during disease acceleration phases [8, 9, 10, 11]. CD8+ T cell mediated targeted cell death involves two principal mechanisms: perforin and Fas-mediated signaling. CD4+ T cell mediated killing occurs via Fas-FasL interactions in MHC class II-

expressing cells such as melanocytes. In a mouse vitiligo model, Lambe T et al showed that perforin knockout mice continued to develop severe vitiligo, whereas, the incidence and severity of vitiligo in mice carrying a mutation in FasL (*gld/gld*) was considerably reduced. These results demonstrate the destruction of melanocytes and the severity of disease are both partially dependent on Fas. They also suggest that CD4+ T cells are sufficient to initiate the destruction of melanocytes, in addition to being adept at providing help to other effector lineages [12].

Recently Li et al, found that a significantly increased risk of vitiligo was associated with the FAS-1377 AA genotype and the FAS-1377 AG genotype when compared with the FAS-1377 GG genotype in Chinese population [13], however these polymorphisms have not been investigated in different ethnic groups. To determine the generality of this association, we studied Fas gene polymorphisms in a Turkish population. In addition to these polymorphisms, our study was the first to investigate the relationship between FAS-124 polymorphism and vitiligo.

Materials and Methods

Subjects: The study was designed as a case controlled study. We examined 112 patients with vitiligo (56 men, 55 women) and 196 healthy control subjects (99 men, 97 women), matched according to age and sex. The mean age of the control subjects was 30.08±13.34 years and of the patients was 35.27±16.71 years. The diagnosis of vitiligo was based on a thorough physical examination, examination with Wood's light (UVA light with a wavelength of 365 nm), and evaluation of the patient's medical history. Also, the clinical types of disease (vitiligo vulgaris or acrofacial vitiligo) were documented. Of the 112 patients, 73 (66%) suffered from vitiligo vulgaris, 39 (34%) acrofacial vitiligo. The control group were chosen from the healthy individuals without any systemic and dermatologic disease. The study was approved by the local ethic committee, and informed consent was obtained from all patients.

Molecular analysis

DNA extraction and analysis: With written informed consent, a blood sample was drawn from each individual. Venous blood samples were collected in ethylenediaminetetra acetic acid (EDTA) contain-

ning tubes. DNA was extracted from whole blood by salting out procedure [14].

Genotypic Analysis of the FAS (CD95, FAS/APO1) -670 G/A (rs 1800682) Polymorphism: W.Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay were used to determine FAS (CD95, FAS/APO1) -670 G/A (rs 1800682) polymorphism. The oligonucleotide primers used to determine the -670 G/A polymorphism in promoter region within the FAS gene were described previously [15, 16]. The primers (GenBank accession no: AY450925), forward 5'-CTACCTAAGAGCTATCTACCGTTC-3'; reverse 5'-GGCTGTCCATGTTGTGGCTGC-3' were used to amplify the 332bp PCR fragment. Polymerase Chain Reaction was performed in a 25 µl volume with 100 ng DNA, 100 µM dNTPs, 20 pmol of each primer, 1.5 mM MgCl₂, 1x PCR buffer with (NH₄)₂SO₄ (Fermentas, Vilnius, Lithuania) and 2U Taq DNA polymerase (Fermentas, Vilnius, Lithuania). Amplification was performed on an automated Thermal Cycler (Techne Flexigene, Cambridge, UK). Polymerase Chain Reaction conditions were 2 min for initial denaturation at 95°C; 35 cycles at 95°C for 45 s for denaturation, 1 min at 62°C for annealing and 90 s at 72°C for extension, followed by 7 min at 72°C for final extension. The PCR products were digested with 10 U MvaI (BstNI Fermentas, Vilnius, Lithuania) at 37°C for 14 hr, the genotyping of the FAS gene was determined by fragment separation at 120 V for 40-50 min on a 3 % Agarose gel containing 0.5µg/ml ethidium bromide. A 100 bp marker (100 bp DNA Ladder, Fermentas) was used as a size standard for each gel lane. The gel was visualized under UV light using a gel electrophoresis visualizing system (Vilber Lourmat). The MvaI restricted products of FAS -670; GG, GA and AA genotypes had band sizes of 184bp/101bp/47bp, 231bp/184bp/101bp/47bp and 231bp/101bp, respectively.

Genotypic Analysis of the FAS (CD95, FAS/ APO1) -1377 G/A (rs2234767) Polymorphism: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay were used to determine FAS -1377 G/A (rs2234767) polymorphism. The oligonucleotide primers used to determine the -1377 G/A polymorphism within the FAS gene were described previously [17, 18]. The primers (GenBank accession no: AY450925), forward 5'-TGTGTGCACAAGGCTGGCGC-3' (mismatch base is underlined); reverse 5'-TGCATCTGTCACTGCACTTACCACCA-3' were used to amplify the 122bp PCR fragment. PCR was performed in a 25 µl volume with 100 ng DNA, 100 µM dNTPs, 20 pmol of each primer, 1.5 mM MgCl₂, 1x PCR buffer with (NH₄)₂SO₄ (Fermentas, Vilnius, Lithuania) and 2U Taq DNA poly-

merase (Fermentas, Vilnius, Lithuania). Amplification was performed on an automated Thermal Cycler (Techne Flexigene, Cambridge, UK). The Bsh1236I restricted products of FAS -1377; GG, GA and AA genotypes had band sizes of 104bp/18bp, 122bp/104bp/18bp and 122bp, respectively.

Genotypic Analysis of the FASL -844 T/C (rs763110) Polymorphism: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay were used to determine FASL -844 T/C (rs763110) polymorphism. The oligonucleotide primers used to determine the -844 T/C polymorphism within the FASL gene were described previously [19, 20]. The primers (GenBank accession no: AF027385, Z96050), forward 5'-CAGCTACTCGGAGGCCAAG-3'; reverse 5'-GCTCTGAGGGGAGAGACCAT-3' were used to amplify the 401bp PCR fragment. PCR was performed in a 25 µl volume with 100 ng DNA, 100 µM dNTPs, 20 pmol of each primer, 1.5 mM MgCl₂, 1x PCR buffer with (NH₄)₂SO₄ (Fermentas, Vilnius, Lithuania) and 2U Taq DNA polymerase (Fermentas, Vilnius, Lithuania). Amplification was performed on an automated Thermal Cycler (Techne Flexigene, Cambridge, UK). The BseMI restricted products of FASL -844; TT, TC and CC genotypes had band sizes of 401bp, 401bp/233bp/168bp and 233bp/168bp, respectively.

Genotypic Analysis of the FASL IVS2nt -124 A/G (rs5030772) Polymorphism: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay were used to determine FASL IVS2nt -124 A/G (rs5030772) polymorphism. The oligonucleotide primers used to determine the IVS2nt -124 A/G polymorphism within the FASL gene were described previously [15, 18]. The primers (GenBank accession no: AF027385, Z96050), forward 5'-GCAGTTCAGACC-TACATGATTAGGAT-3' (mismatch base is underlined); reverse 5'-CCAGATACAGACCTGTTAAATGGGC-3' were used to amplify the 219bp PCR fragment. PCR was performed in a 25 µl volume with 100 ng DNA, 100 µM dNTPs, 20 pmol of each primer, 1.5 mM MgCl₂, 1x PCR buffer with (NH₄)₂SO₄ (Fermentas, Vilnius, Lithuania) and 2U Taq DNA polymerase (Fermentas, Vilnius, Lithuania). Amplification was performed on an automated Thermal Cycler (Techne Flexigene, Cambridge, UK). The BseGI restricted products of FASL IVS2nt -124 A/G; AA, AG and GG genotypes had band sizes of 219bp, 219bp/190bp/29bp and 190bp/29bp, respectively. All procedures were conducted in a manner blind to the case status and other characteristics of the participants. Scoring of gels and data entry were conducted independently by two persons. We performed the PCRs and evaluated the results without knowing the groups of the

subjects. At least 10% of the samples were retested, and the results were 100% concordant.

Statistical Tests

All statistical tests were carried out using MedCalc Software Verrision 12.3.0 (1993-2012 Broekstraat 52, 9030 Mariakerke, Belgium). All values were presented as mean ± standard deviation (SD). The strength of association was estimated by calculating the odds ratios (ORs) with 95% confidence intervals (95% CIs), which were generated by logistic regression analysis. To calculate the alignment of distribution of alleles and genotypes (*Hardy-Weinberg* equilibrium), chi-square test was applied. Potential risks of genotypes and alleles were determined by calculating the Odds ratio. The relationship between groups and gender was analyzed using chi-square test. P values <0.05 were considered statistically significant.

Results

As a result of the *Hardy-Weinberg* equilibrium control, all polymorphisms were stable in the control groups (p>0,05). Except for FAS 670 patients group (p<0,05), the other polymorphisms were in the *Hardy Weinberg* equilib-

rium (p>0,05). When calculations of FAS 670 and FAS 1377 polymorphisms were performed, GG genotype was taken as a reference. For FASL-844, TT genotype and for FASL, 124 AA genotype was taken as reference. As shown in **Table 1**, the frequencies of FAS-670 genotypes was statistically significant between patients and controls (p=0,0035). This difference come from GG (p=0,019) and GA (p=0,0172) groups. In order to interpret the odss ratio, the results must be statistical significant. For FAS-670, OR was statistically significant between GG and GA (p=0,0052). GA genotype was 2.874 times more frequent in patients than in controls (OR=2,874 ; 95%CI, 1,37-6,03, p=0,0052). The other ORs couldn't interpret, since there weren't statistically significant results (p>0,05). According to allele frequencies, patient and control groups were similar (p=0,4159). As shown in **Table 2**, the frequencies of FAS-1377 genotypes were not statistically significant between patients and controls (p=0,5812). The ORs results could not interpret, since there weren't statistically significant (p>0,05). As shown in **Table 3**, the frequencies of FAS-844 genotypes were not statistically significant between

Table 1. Genotypic Frequencies of FAS-670 Polymorphisms in Patients and Controls and Their Associations with Risk of Vitiligo

FAS 670	Controls (n=196)		Patients (n=113)		Chi-square	OR
Genotype	n	%	n	%	p	OR(%95 CI) p
GG	41	20,9	11	9,8	0,0035	1,00 Reference
GA	83	42,4	64	57,2		2,874 (1,37-6,03) 0,0052
AA	72	36,7	38	33,0		1,915 (0,88-4,16) 0,1001
HW (p)	0,066		0,028			
Allel Frequency	n	%	n	%		
G	165	42,1	86	38,4	0,4159	----
A	227	57,9	140	61,6		1,166 (0,83-1,63) 0,3689

Table 2. Genotypic Frequencies of FAS-1377 Polymorphisms in Patients and Controls and Their Associations with Risk of Vitiligo

FAS 1377	Controls (n=196)		Patients (n=113)		Chi-square	OR
Genotype	n	%	n	%	p	OR(%95 CI) p
GG	138	70,4	78	69,0	0,5812	1,00 Reference
GA	51	26,0	33	29,2		1,145 (0,68-1,92) 0,6092
AA	7	3,6	2	1,8		0,505 (0,10-2,49) 0,4021
HW (p)	0,405		0,480			
Allel Frequency	n	%	n	%		
G	325	83,6	199	83,4	0,9643	----
A	68	16,4	37	16,6		1,015 (0,65-1,58) 0,9460

Table 3. Genotypic Frequencies of FAS-844 Polymorphisms in Patients and Controls and Their Associations with Risk of Vitiligo

FASL 844	Controls (n=196)		Patients (n=113)		Chi-square	OR	
Genotype	n	%	n	%	p	OR(%95 CI)	p
TT	46	23,5	31	27,4	0,6956	1,00	Reference
TC	99	50,5	56	49,6		0,839 (0,48-1,47)	0,5407
CC	51	26,0	26	23,0		0,756 (0,39-1,46)	0,4045
HW (p)	0,8791		0,9415				
Allel Frequency	n	%	n	%			
T	191	48,7	118	52,2	0,4522	----	
C	201	51,3	108	47,8		0,869 (0,63-1,21)	0,4037

Table 4. Genotypic Frequencies of FAS-124 Polymorphisms in Patients and Controls and Their Associations with Risk of Vitiligo

FAS 124	Controls (n=196)		Patients (n=113)		Chi-square	OR	
Genotype	n	%	n	%	p	OR(%95 CI)	p
AA	134	68,4	78	69,0	0,6233	1,00	Reference
GA	57	29,1	30	26,5		0,90 (0,54-1,52)	0,7057
GG	5	2,5	5	4,5		1,72 (0,48-6,12)	0,4039
HW (p)	0,7145		0,3456				
Allel Frequency	n	%	n	%			
A	325	82,9	186	82,3	0,9348	----	
G	67	17,1	40	17,7		1,04 (0,68-1,60)	0,8476

patients and controls (p=0,6956). The ORs results couldn't interpret, since there weren't statistically significant (p>0,05). As shown in **Table 4**, the frequencies of FAS-124 genotypes were not statistically significant between patients and controls (p=0,6233). The ORs results couldn't be interpreted, since they were not statistically significant (p>0,05).

Correlations between clinical types of vitiligo (vitiligo vulgaris and acrofacial vitiligo) and different polymorphisms have been done. No differences in FAS-124, FAS-670, FAS-844 ve FAS 1377 polymorphisms were detected between vitiligo vulgaris and acrofacial vitiligo.

Discussion

The highest risk of the vitiligo has been recorded in patients with autoimmune diseases and also positive family history of vitiligo [11]. In this study, we explored the association of the FAS 670, FAS 1377, FASLG 844 and FASL 124 polymorphisms with the risk of vitiligo in a Turkish population. The FAS 1377, FASLG 844 and FASL 124 polymorphisms did not affect the risk of vitiligo. However, we found that a increased risk of vitiligo risk ap-

peared to be associated with FAS 670GA. To the best of our knowledge, this is the first study to investigate whether or not FAS and FASLG polymorphisms are associated with a risk of vitiligo in Turkish population. Published association studies of FAS polymorphisms and autoimmune disease risk include *Sjögren's syndrome*, systemic lupus erythematosus, autoimmune hepatitis, alopecia areata and *Guillain-Barré syndrome* [21, 22, 23, 24 ,25]. Fas-FasL signaling has also been implicated in other forms of cell-specific autoimmunity including experimental autoimmune encephalomyelitis and autoimmune diabetes [26, 27].

Apoptosis, or programmed cell death, is an important mechanism used to delete autoreactive lymphocytes and may be a key factor in the development of autoimmunity. In addition, increased expression of Fas, Fas ligand (FasL), and increased apoptosis may play a part in the observed cutaneous melanocyte destruction although this remains controversial [28]. Redistribution of the normally intracellular Ro and La autoantigens to surface membrane blebs on apoptotic cells has recently been demonstrated, a process by

which these autoantigens may be accessible to the immune system. It is therefore conceivable that apoptosis may underlie the induction and facilitation of Ro/La autoantibody responses in primary Sjögren's syndrome. After apoptosis, cell corpses are rapidly recognized and phagocytosed by professional phagocytes, such as macrophages and dendritic cells. The rapid removal of cell corpses by phagocytes prevents the release of potentially toxic or immunogenic materials from dying cells [29]. Vitiligo is an acquired melanocyte loss disorder resulting from an immunologically mediated attack on melanocytes, and autoimmunity may play a part in its pathogenesis. It is suspected to be a T cell-mediated autoimmune disease of the melanocytes, where Fas is expressed on melanocytes and FasL on perilesional infiltrates. Vitiligo is an autoimmune disease however the mechanism behind tolerance breakdown of the self-peptides remains unclear [30, 31]. Similarly, it is therefore conceivable that apoptosis may underlie the induction and facilitation of autoimmune responses in vitiligo.

Li et al studied functional polymorphisms of the FAS and FASLG genes in 750 vitiligo patients and 756 controls, all of Han Chinese descent, with respect to the FAS-1377 G>A, FAS-670 A>G, and FASLG-844 T>C polymorphisms. Their ORs (95% CI) for vitiligo of FAS-1377 AA and AG genotypes were 1.49 (1.07-2.08) and 1.31 (1.05-1.63). They observed a significant increase in the frequency of the AA and AG allele in vitiligo patients as compared with that of the FAS-1377 GG groups. They found that a significantly increased risk of vitiligo was associated with the FAS-1377 AA genotype (adjusted OR, 1.49; 95% CI, 1.07-2.08) and the FAS-1377 AG genotype (adjusted OR, 1.31; 95% CI, 1.05-1.63) when compared with the FAS-1377 GG genotype. They suggested that allelic variance in the FAS gene itself or by other genes in linkage disequilibrium with this gene could predispose to the development of vitiligo [13]. It can be hypothesized that apoptosis can cause the presentation to the immune system. In vitiligo, however, T cell tuning allows T cells with higher affinity for melanocyte differentiation antigens to enter the circulation after escaping clonal deletion in primary lymphoid organs. The resulting efficacious and progressive autoimmune response to melanocytes

provides a roadmap for melanoma therapy. This is the first report of an association of Fas gene polymorphism and vitiligo in a Caucasian population. This is the also first study of an association between FAS-124 gene polymorphism and vitiligo. The results of this study suggest that the presence of FAS-670 GA genotype can be regarded as risk factor for vitiligo, and especially for vitiligo in Turkish population. The influence of a functional single-nucleotide polymorphism at position -670 in the promoter of the apoptosis gene FAS on susceptibility to autoimmune diseases needs further investigations. Although it has not yet been possible to assign any particular allele or genotype to the control of FAS expression, this polymorphism has been described to be associated with several autoimmune diseases including lupus erythematosus [22]. Our finding suggests that apoptosis may contribute to development of autoimmune reactions and that FAS function might be relevant for vitiligo.

In view of these data the involvement of other genetic and/or environmental factors seems to be required and to be more important in vitiligo. Further analysis of other FAS and cytokine polymorphisms and extended samples may clarify the current findings, eventually resulting in a better understanding of the genetic and immunologic components of vitiligo.

References

1. Le Poole C, Boissy RE. Vitiligo. *Semin Cutan Med Surg* 1997; 16: 3-14. PMID: 9125760
2. Ongenaes K, Van Geel N, Naeyaert JM. Evidence for an autoimmune pathogenesis of vitiligo. *Pigment Cell Res* 2003; 16: 90-100. PMID: 12622785
3. Wankowicz-Kalinska A, Le Poole C, van den Wijngaard R, Storkus WJ, Das PK. Melanocyte-specific immune response in melanoma and vitiligo: two faces of the same coin? *Pigment Cell Res* 2003; 16: 254-260. PMID: 12753398
4. Alkhateeb A, Fain PR, Thody A, Bennett DC, Spritz RA. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. *Pigment Cell Res* 2003; 16: 208-214. PMID: 12753387
5. Yazici AC, Erdal ME, Kaya TI, et al. Lack of association with TNF-alpha-308 promoter polymorphism in patients with vitiligo. *Arch Dermatol Res* 2006; 298: 46-49. PMID: 16691430
6. Ruiz-Argüelles A, Brito GJ, Reyes-Izquierdo P, Pérez-Romano B, Sánchez-Sosa S. Apoptosis of melanocytes

- tes in vitiligo results from antibody penetration. *J Autoimmun* 2007; 29: 281-286. PMID: 17888626.
7. Sandoval-Cruz M, Garcia-Carrasco M, Sánchez-Porras R, et al. Immunopathogenesis of vitiligo. *Autoimmun Rev* 2011; 10: 762-765. PMID: 21334464
 8. Palermo B, Campanelli R, Garbelli S, et al. Specific cytotoxic T lymphocyte responses against Melan-A/MART1, tyrosinase and gp100 in vitiligo by the use of major histocompatibility complex/peptide tetramers: the role of cellular immunity in the etiopathogenesis of vitiligo. *J Invest Dermatol* 2001; 117: 326-332. PMID: 115113119.
 9. Le Poole IC, Luiten RM. Autoimmune etiology of generalized vitiligo. *Curr Dir Autoimmun*. 2008;10:227-43
 10. Das PK, van den Wijngaard RM, Wankowicz-Kalinska A, Le Poole IC. A symbiotic concept of autoimmunity and tumour immunity: lessons from vitiligo. *Trends Immunol* 2001; 22: 130-136. PMID: 11286727
 11. Le Poole IC, Wańkowicz-Kalińska A, van den Wijngaard RM, Nickoloff BJ, Das PK. Autoimmune aspects of depigmentation in vitiligo. *J Invest Dermatol Symp Proc* 2004; 9: 68-72. PMID: 14870989
 12. Lambe T, Leung JC, Bouriez-Jones T, et al. CD4 T cell-dependent autoimmunity against a melanocyte neoantigen induces spontaneous vitiligo and depends upon Fas-Fas ligand interactions. *J Immunol* 2006; 177: 3055-3062.
 13. Li M, Sun D, Li C, et al. Functional polymorphisms of the FAS gene associated with risk of vitiligo in Chinese populations: a case-control analysis. *J Invest Dermatol* 2008; 128: 2820-2824. PMID: 18548110
 14. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16: 1215. PMID: 3344216
 15. Fuks A, Parton LA, Polavarapu S, et al. Polymorphism of Fas and Fas ligand in preterm premature rupture of membranes in singleton pregnancies. *Am J Obstet Gynecol* 2005; 193: 1132-1136. PMID: 16157125
 16. Aguilar-Reina J, Ruiz-Ferrer M, Pizarro MA, Antiñolo G. The -670A > G polymorphism in the promoter region of the FAS gene is associated with necrosis in periportal areas in patients with chronic hepatitis C. *J Viral Hepat* 2005; 12: 568-573. PMID: 16255757
 17. Li C, Wu W, Liu J, et al. Functional polymorphisms in the promoter regions of the FAS and FAS ligand genes and risk of bladder cancer in south China: a case-control analysis. *Pharmacogenet Genomics* 2006; 16: 245-251. PMID: 16538171
 18. Li C, Larson D, Zhang Z, et al. Polymorphisms of the FAS and FAS ligand genes associated with risk of cutaneous malignant melanoma. *Pharmacogenet Genomics* 2006; 16: 253-263. PMID: 16538172
 19. Zhang X, Miao X, Sun T, et al. Functional polymorphisms in cell death pathway genes FAS and FASL contribute to risk of lung cancer. *J Med Genet* 2005; 42: 479-484. PMID: 15937082
 20. Sun T, Miao X, Zhang X, Tan W, Xiong P, Lin D. Polymorphisms of death pathway genes FAS and FASL in esophageal squamous-cell carcinoma. *J Natl Cancer Inst* 2004; 96: 1030-1036. PMID: 15240787
 21. Badillo-Almaraz I, Avalos-Diaz E, Villalobos-Hurtado R, Herrera-Esparza R. FasL and Bax genes are differentially expressed in acinar epithelium and inflammatory cells of primary Sjögren salivary glands. *Minerva Med* 2003; 94: 341-345. PMID: 14973428
 22. Molin S, Weiss EH, Ruzicka T, Messer G. The FAS/cd95 promoter single-nucleotide polymorphism -670 A/G and lupus erythematosus. *Clin Exp Dermatol* 2012; 37: 425-427. PMID: 22299671
 23. Fan X, Shangguan L, Li M, Li CY, Liu B. Functional polymorphisms of the FAS/FASLG genes are associated with risk of alopecia areata in a Chinese population: a case-control analysis. *Br J Dermatol* 2010; 163: 340-344. PMID: 20394629
 24. Agarwal K, Czaja AJ, Donaldson PT. A functional Fas promoter polymorphism is associated with a severe phenotype in type 1 autoimmune hepatitis characterized by early development of cirrhosis. *Tissue Antigens* 2007; 69: 227-235. PMID: 17493146
 25. Geleijns K, Laman JD, van Rijs W, et al. Fas polymorphisms are associated with the presence of anti-ganglioside antibodies in Guillain-Barre syndrome. *J Neuroimmunol* 2005; 161: 183-189. PubMed PMID: 15748958
 26. Dittel BN, Merchant RM, Janeway CA Jr. Evidence for Fas-dependent and Fas-independent mechanisms in the pathogenesis of experimental autoimmune encephalomyelitis. *J Immunol* 1999; 162: 6392-6400. PMID: 10352252
 27. Vence L, Benoist C, Mathis D. Fas deficiency prevents type 1 diabetes by inducing hyporesponsiveness in islet beta-cell-reactive T-cells. *Diabetes* 2004; 53: 2797-2803. PMID: 15504959
 28. Ogawa N, Dang H, Talal N. Apoptosis and autoimmunity. *J Autoimmun* 1995; 8: 1-19. PMID: 7734031
 29. Ohlsson M, Jonsson R, Brokstad KA. Subcellular redistribution and surface exposure of the Ro52, Ro60 and La48 autoantigens during apoptosis in human ductal epithelial cells: a possible mechanism in the pathogenesis of Sjogren's syndrome. *Scand J Immunol* 2002; 56: 456-469.
 30. Van den Wijngaard R, Wankowicz-Kalinska A, Le Poole C, Tigges B, Westerhof W, Das P. Local immune response in skin of generalized vitiligo patients. Destruction of melanocytes is associated with the prominent presence of CLA+ T cells at the perilesional site. *Lab Invest* 2000; 80: 1299-1309. PMID: 10950121
 31. Le Poole IC, van den Wijngaard RM, Westerhof W, Das PK. Presence of T cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. *Am J Pathol* 1996; 148: 1219-1228. PMID: 8644862