Int J Cancer Manag. 2017 September; 10(9):e10528. doi: 10.5812/ijcm.10528.

Published online 2017 September 30. **Research Article**

The Relationship of *Fas* Promoter Polymorphisms and Breast Cancer Risk in North-West of Iran: A Haplotype and in Silico Analysis

Narges Dastmalchi,[1] Reza Safaralizadeh,[1] Mohammad Ali Hosseinpour Feizi,[1,*] and Nasser Pouladi[2]

[1]Animal Biology Department, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
[2]Cellular and Molecular Biology Department, Faculty of Biology, Azarbaijan Shahid Madani University, Tabriz, Iran

[*]*Corresponding author*: Mohammad Ali Hosseinpour Feizi, Sciences and Technology Department, Azadi blv, Tabriz, Iran. Tel/Fax: +98-4133370497, E-mail: pourfeizi@eastp.ir

Received 2017 January 16; **Revised** 2017 March 15; **Accepted** 2017 August 26.

Abstract

Background: It is better that the haplotypes and combined genotypes of *fas* molecular markers to be studied rather than each polymorphism, independently.
Objectives: The present study aims at investigating whether the *fas*-1377G/A (rs2234767),-670A/G (rs1800682) polymorphisms as well as their combined genotypes and haplotypes affect the risk of developing breast tumors.
Methods: This study was conducted on 200 Iranian patients with breast cancer and 186 controls. The *fas*-1377G/A,-670A/G polymorphisms were determined, using tetra-ARMS-PCR and RFLP-PCR, respectively. The java stat online statistics package and *SHEsis* programs were used for data analysis. PNI modeler program was applied for in silico analysis.
Results: The results of the present study did not show any significant differences in the genotype distribution of *fas* rs2234767, rs1800682 as well as their haplotypes and genotype combinations between cases and controls. Furthermore, in silico analysis indicated that *fas*-1377,-670 polymorphisms change the interaction of proteins with the promoter of *fas*.
Conclusions: The findings of this study proposed that the *fas* promoter variants did not have a major effect on the risk of breast cancer. Further studies are required to validate these conclusions across various populations. Based on in silico analysis, there was an altered binding pattern of proteins to the promoter of *fas* around the polymorphisms.

Keywords: Breast Cancer, Haplotype, In Silico Analysis, *Fas* Polymorphism

1. Background

Breast cancer is one of the most frequent cancers (1) and the main cause of cancer-related deaths (14%) among women all over the world (2-4). In addition to environmental agents, body weight (2) and lifestyle factors, such as alcohol consumption, physical inactivity, smoking, (5) and genetic background may have an important effect in developing breast tumors (5, 6). Researchers have demonstrated that, in addition to unlimited cell proliferation, the suppression of apoptosis correlated with the development of various tumors (3). Apoptosis, significantly, regulates various critical cellular and biological processes, such as organ development, homeostasis, and tumor cells destruction (7, 8). Abnormal regulation of apoptotic pathways and consequent deregulation of mentioned biological processes lead to the development of human malignancies (3). Death receptor on the surface of many cell types, *fas* and its ligand, *fasL* as members of the tumor necrosis factor superfamily (TNF), interact to initiate the extrinsic death signal pathway, which consequently leads to apoptotic cell death (7, 9-12). Structural alterations of *fas* and *fasL* may influence the expression of them and consequently result in the development of various tumors, such as breast tumors (7, 13). The human *fas* on 10q24.1 (12, 14), which consists of 9 exons and 8 introns, (14) has 2 single nucleotide polymorphisms (SNPs) in its promoter. One of them is a G-A transition at nucleotide number -1377 (*fas*-1377G > A) in the silencer region, and the other is an A-G transition at nucleotide number -670 (*fas*-670A > G) in the enhancer site. By electrophoretic mobility shift assay (EMSAs), the previous studies reported that reduced binding of Sp1 and STAT1 has been observed, respectively, in the presence of *fas*-1377A and *fas*-670G alleles, which may decrease transcription and translation of the *fas* (2, 15). Some studies showed that *fas* promoter SNPs correlated with various malignancies, such as breast cancer (2, 16). Few surveys investigated the correlation between *fas* promoter polymorphisms and breast cancer risk. Hence, the mentioned association is almost unknown. To investigate the effect of *fas* promoter polymorphisms on the risk of breast cancer, we genotyped the *fas*-1377G > A,

fas-670A > G substitutions and evaluated their association with breast cancer risk in northwest of Iran. Moreover, in the present study, in silico application was used in order to detect the alterations of protein binding pattern in the *fas* sequence around SNPs.

2. Methods

2.1. Patients with Cancer and Healthy Controls

This case-control study was performed on 200 women with breast cancer and 186 healthy women without cancer history among their relatives. All of the patients had undergone mastectomy/lumpectomy at Imam Reza and Noor-E-Nejat Hospitals in Tabriz, Iran between 2008 and 2012. All participants were from northwest provinces of Iran. In the current study, northwest of Iran includes 4 provinces, including East Azarbaijan, West Azarbaijan, Ardabil, and Kurdistan, according to the official map of Iran. The extraction of DNA was performed on all subjects' peripheral blood samples, using SDS/proteinase K and salting-out method (17). We did not use DNA extraction kit in this study. Blood drawing and DNA extraction were performed with consent from all subjects as well as permit number 5.4.3259/13.3.92 (2013) from the 13th ethics committee of Tabriz University of Medical Sciences research center, which were concordant with the Helsinki declaration. The clinicopathological characteristics were obtained from medical records. Tumor-Node-Metastasis (TNM) staging was applied for tumor staging (18).

2.2. Primers Selection and PCR Amplification

Genotyping of *fas*-1377G > A and *fas*-670A > G was done, using tetra-amplification refractory mutation system-polymerase chain reaction (Tetra-ARMS-PCR) and restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) (19), respectively.

2.3. Tetra-ARMS-PCR

Sequences of the primers were designed according to the previous studies (2); amplicon sizes are shown in Table 1. The amplification of both wild-type and polymorphic alleles was, simultaneously, conducted in a single-tube PCR. In order to detect *fas*-1377G > A, PCR amplification was prepared in a total volume of 25 μL containing: 1 μL template DNA (average concentration of 200 ng), 0.8 μL of each primer (10 pM), 2.5 μL of reaction buffer 10x, 0.5 μL of deoxyribonucleotide triphosphate (dNTP), 0.85 μL of $Mgcl_2$, and 0.2 μL of Taq DNA polymerase (Cinnagenn, Tehran, Iran). PCR program was run at 95°C for 5 minutes, followed by 30 cycles of a denaturation for 30 seconds at 95°C, annealing for 30 seconds at 64°C, extension for 30 seconds at 72°C, and a final extension for 10 minutes at 72°C in a thermo-cycler (Sensoquest, GmbH, Germany). Amplicon sizes for G and A alleles were 216 bp and 340 bp, respectively. The products were analyzed by gel electrophoresis on a 2% agarose gel containing ethidium bromide.

Table 1. Tetra-ARMS-PCR/RFLP-PCR Primers for *fas*-1377G/A (rs2234767), *fas*-670A/G (rs1800682), Respectively

Primers	Sequence (5' → 3')	dbSNP
Forward outer primer (FO)	5'-CCTTCCCTCACACCCCTTTTCCTTCC-3'	rs2234767
Reverse outer primer (RO)	5'-CTTTGGCATCGTCCACCAAGCTCT-3'	
Forward inner primer (FI), (A allele)	5'-AGTGTGTGCACAAGGCTGGCCCA-3'	
Reverse inner primer (RI), (G allele)	5'-TTAGTGCCATGAGGAAGACCCTGT-3'	
Forward primer (F)	5'-ATAGCTGGGGCTATGCGATT-3'	rs1800682
Reverse primer (R)	5'-CATTTGACTGGGCTGTCCAT-3'	

2.4. RFLP-PCR

The *fas*-670A > G genotypes were determined, using RFLP-PCR. Sequences of the primers, as described earlier, (20) are shown in Table 1. Primers for this SNP produce a 193 bp fragment. Amplification was done under the following conditions: A 25 μL reaction mixture contained 2.5 μL of reaction buffer 10x, 1 μL of DNA, 0.9 μL of each primer (10 pM), 0.5 μL of dNTP, 0.76 μL of $Mgcl_2$, and 0.18 μL of Taq. The enzyme, Bme1390I (ScrFI) (Fermentas, USA), was used to distinguish the *fas*-670A > G. ScrFI digestion generated the following fragments: *fas*-670A allele, a single fragment of 193 bp; and *fas*-670G, fragments of 136 bp, and 57 bp (gain of ScrFI digestion site).

2.5. In Silico Analysis

PNI modeler, as an online web program (available on http://165.246.44.34/PNI modeler/), predicts nucleotides that bind to proteins. This application was used to indicate whether the *fas*-1377G/A and *fas*-670A/G influence the protein binding sites in the promoter of *fas*.

2.6. Statistical Analysis

Genotypes and alleles distributions were compared between patients and controls by Pearson's Chi-square or Fisher's exact tests. The strength of the association

between the polymorphisms and breast cancer was obtained by odds ratios (ORs) with 95% confidence intervals (95% CIs). The java stat online statistics package (available on http://statpages.org/ctab2x2.html) was used to calculate mentioned statistical tests. Haplotype analysis was performed, using *SHEsis* software (available on http://analysis.bio-x.cn/myAnalysis.php). The *SHEsis* software was also used to calculate the Linkage disequilibrium (D') and correlation coefficient (r^2) between 2 polymorphic sites and check Hardy-Weinberg equilibrium (HWE) in controls based on Pearson's Chi-square test. For all examinations, P value < 0.05 was a significant result. The mean values were calculated by statistical package for the social sciences (SPSS) software (v.16). Based on Bonferroni correction test, adjusted P value reported would be 0.005.

3. Results

3.1. Subjects' Characteristics

The average standard deviation age was 47.93 ± 10.03 for cases and 42.49 ± 12.51 for controls; all participants were women. Clinical characteristics of the patients are indicated in Table 2. According to clinicopathological information, 9 patients had ductal carcinoma in situ (DCIS), 181 of them had invasive ductal carcinoma (IDC), and 10 patients had invasive lobular carcinoma (ILC).

Table 2. Characteristics of Controls and Patients with Breast Cancer[a]

Characteristics	Cases	Controls
Mean age ± standard deviation	47.93 ± 10.03	42.49 ± 12.51
Range of age (year)	25 - 81	19 - 79
Tumor-type		
DCIS	9 (4.5)	-
IDC	181 (90.5)	-
ILC	10 (5)	-
Tumor-stage		
Stage 0	13 (6.5)	-
Early (I and II)	85 (42.5)	-
Late (III and IV)	94 (47)	-
Unknown	8	
Tumor grade		
I	38 (19)	-
II	122 (61)	-
III	20 (10)	-
Unknown	20	
Lymph-node metastases		
Positive	114 (57)	-
Negative	76 (38)	-
Unknown	10	
Tumor-size		
≤ 3.9	98 (49)	-
> 3.9	91 (45.5)	-
Unknown	11	
Side-involved		
Right	95 (47.5)	-
Left	94 (47)	-
Both	11 (5.5)	-

[a]Values are expressed as No. (%).

3.2. Association of Fas Polymorphisms and Breast Cancer

The allele/genotype distributions of *fas*-1377G/A,-670A/G in patients and controls are shown in Table 3. For *fas*-1377G > A, the GG genotype had a higher frequency in controls rather than patients (65.6% and 60%, respectively). In contrast, the frequency of GA genotype in patients was 7% higher than controls (OR = 1.338; 95% CI = 0.854 - 2.097; P = 0.181). For *fas*-670A > G, homozygous genotype for A allele showed 7.47% higher frequency in controls than cases, while heterozygous genotype had more frequency among patients (OR = 1.408; 95% CI = 0.895 - 2.217; P = 0.118). The distribution of the *fas*-1377GG, GA, AA, and *fas*-670AA, AG, GG genotypes among patients was not significantly different from those among the controls (P > 0.05). Genotype distribution of *fas*-1377A > G/-670G > A in controls was in consistence with Hardy-Weinberg equation (HWE) (P > 0.05). Thus, observed genotype distribution in healthy subjects represented the genotype frequency in the overall northwest population of Iran. Allelic frequencies were also calculated for each polymorphism. The allele frequencies for *fas*-1377A and *fas*-670G were 19.09% and 31.72% in controls compared with 21.25% and 35.5%, in patients, the frequencies for *fas*-1377G and *fas*-670A were 80.91% and 68.28% in controls compared with 78.75% and 64.5% in patients, respectively. The observed differences for frequency of alleles between cases and controls were not significant (P > 0.05). Eight combined genotypes of *fas* were obtained in patients and healthy subjects (Table 4). The most frequent combined genotype in both groups was *fas*-1377GG/-670AA (Δ = 7.7%). Because 2 polymorphisms in *fas* (-1377G > A/-670A > G) were in linkage disequilibrium with each other (D' = 0.798, r^2 = 0.318), the combined association of them with breast cancer risk was calculated by haplotype analysis. The frequency of *fas* (-1377/-670) haplotypes did not show a significant difference between cases and controls (P >

0.05) (Table 4).

3.3. In Silico Analysis

Based on PNI modeler results (Figure 1, i, ii), the *fas*-1377G > A and *fas*-670A > G altered protein interaction pattern of the promoter sequence around both polymorphisms that might also alter transcription factor binding to the promoter of *fas* and might change gene transcription and translation. For *fas*-1377G > A, PNI modeler revealed that there was 1 shift to the left for probable protein interaction site in upstream region of nucleotide -1377G (i). Moreover, there were 2 lost protein interaction sites around nucleotide -1377G. For -1377G > A and -670A > G, 2 (i) and 1 (ii) interaction sites vanished respectively, that both of them were seen in complementary DNA sequence.

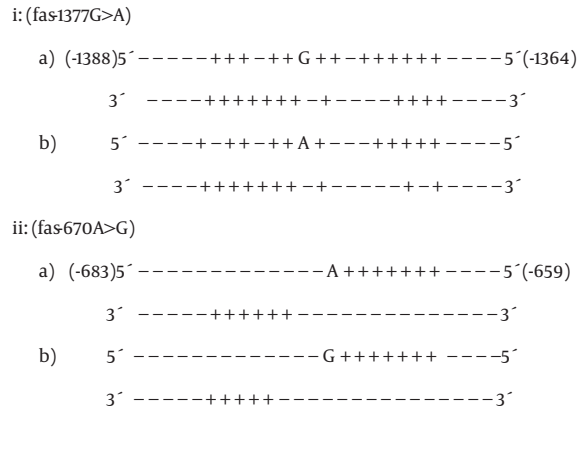

Figure 1. PNI Modeler Prediction Results, - and + signs determine non-interaction and protein interaction nucleotides, respectively. Polymorph alleles are shown in bold face. *Fas* DNA duplex that have G nucleotide in site -1377 (i a); *fas* DNA duplex with exchanged nucleotide A in site -1377 (i b); *fas* DNA duplex that have A nucleotide in site -670 (ii a); *fas* DNA duplex with exchanged nucleotide G in site -670 (ii b).

4. Discussion

Death receptor and its ligand interact to initiate the extrinsic apoptotic pathway that in some cell types links to the intrinsic pathway of apoptosis through Bid protein (20). The *fas* is expressed on the tumor cells that can be assistant to the *Fas*-triggered destruction of tumor cells by the immune system. Some studies showed that the *fas* expression was decreased on breast tumor cells; this event may be due to the *fas* polymorphisms (2, 21). Structural changes of *fas*, including 2 common polymorphisms of its promoter (2) were seen in various human malignancies, such as T-cell leukemia, (22) urinary bladder, (23) non-small cell lung cancer (24), and malignant melanoma, (25) as well as breast cancer (2) and autoimmune diseases, such as rheumatoid arthritis (26). To the best of the authors' knowledge, as the first case-control study in northwest of Iran, we investigated the correlation between 2 common polymorphisms in the promoter of *fas*. The present study did not show any significant association between the risk for breast cancer and *fas*-670A > G. The results of studies on breast cancer in Chinese population (3) and prostate cancer in Portuguese population (27) revealed that the *fas*-670GG genotype had a protective effect on the development of breast and prostate cancers. Recently, Xu et al. applied a meta-analysis of cancer cases and controls, including *fas*-1377G > A and *fas*-670A > G from 52 case-control studies, with similar results to the present study; the results showed no significant association between *fas*-670A > G and breast cancer risk that might have variability based on various tumor types, examined population, and different technics in genotyping assay. Therefore, this explanation may be a reason for different observed results, even conflicting, among various investigations (28). Several studies have analyzed the correlation between *fas*-1377G > A and human cancers. Studies on childhood acute lymphoblastic leukemia (15) and hepatitis B virus infection (29) in Iran have shown no significant association in *fas*-1377G > A and *fas*-670A > G with the risk of both diseases that were in agreement with the findings of this study. Some of the studies have demonstrated that the A allele has a higher frequency among cases than healthy subjects, (5, 12); however, contrastingly in some others, the A allele had a higher frequency in controls in comparison with patients (8, 16, 30). In the present study, the frequency of *fas*-1377A allele and GA genotype was higher in patients rather than the control group, but the differences were not significant. Several meta-analyses have examined the relationship between *fas*-1377G > A and breast cancer risk. Some of them have demonstrated that the *fas*-1377G > A polymorphism is associated with higher sensitivity for breast cancer (28, 31, 32). Turkish group's investigation revealed that homozygosity for *fas*-1377G was associated with higher risk of bladder cancer (33). Crew et al. reported that the *fas*-1377G > A showed no different frequency between breast cancer patients and controls in Long Island, New York (16). Hashemi et al. also observed the same result in south of Iran (2). The results of the present study were in agreement with the results of the survey conducted by Crew et al. (16) and Hashemi et al. (2). Several investigations have shown the correlation of *fas* promoter haplotypes with the cancer development risk among various ethnic groups. Zhang et al. reported a significant more frequency for (-1377A-670A) haplotype among patients rather than controls, whereas their study showed a higher frequency for (-1377G-670G) haplotype among controls in comparison with patients

Table 3. Frequency Distribution of *fas*-1377G > A and *fas*-670A > G Polymorphisms in Controls and Breast Cancer Patients, Global Minor Allele Frequency (MAF) for rs2234767, and rs1800682 is 0.18 and 0.4, Respectively

Polymorphisms	Breast Cancer: No. (%)	Control: No. (%)	OR (95%CI)	P Value[a]
***fas*-1377A > G**				
GG	120 (60)	122 (65.6)	Ref	-
GA	75 (37.5)	57 (30.64)	1.338 (0.854 - 2.097)	0.181
AA	5 (2.5)	7 (3.76)	0.726 (0.194 - 2.638)	0.592
GA + AA	80 (40)	64 (34.40)	1.271 (0.822 - 1.965)	0.256
Alleles				
G	315 (78.75)	301 (80.91)	Ref	-
A	85 (21.25)	71 (19.09)	1.144 (0.792 - 1.653)	0.454
***fas*-670G>A**				
AA	84 (42)	92 (49.47)	Ref	-
AG	90 (45)	70 (37.63)	1.408 (0.895 - 2.217)	0.118
GG	26 (13)	24 (12.9)	1.187 (0.604 - 2.332)	0.594
AG + GG	116 (58)	94 (50.5)	1.352 (0.886 - 2.062)	0.141
Alleles				
A	258 (64.5)	254 (68.28)	Ref	-
G	142 (35.5)	118 (31.72)	1.185 (0.868 - 1.616)	0.267

Abbreviations: CI, Confidence Interval; OR, Odds Ratio.
[a] x^2-test.

Table 4. Frequency Distribution of *fas* Genotype Combinations and *fas* Pairwise Haplotypes in Cases and Controls

fas-1377G > A	*fas*-670A > G	Cases No. (%) (N = 200)	Controls, No. (%), (N = 186)	OR (95% CI)	P Value
G/G	A/A	76 (38)	85 (45.7)	Ref	-
G/G	A/G	27 (13.5)	28 (15.06)	1.078 (0.559 - 2.081)	0.809
G/G	G/G	17 (8.5)	9 (4.84)	2.113 (0.828 - 5.487)	0.085
G/A	A/A	8 (4)	7 (3.76)	1.278 (0.397 - 4.147)	0.649
G/A	A/G	61 (30.5)	42 (22.58)	1.505 (0.763 - 2.977)	0.205
G/A	G/G	6 (3)	8 (4.30)	0.839 (0.245 - 2.821)	0.755
A/A	A/G	2 (1)	0	-	0.227
A/A	G/G	3 (1.5)	7 (3.76)	0.479 (0.094 - 2.158)	0.344
Pairwise haplotypes		Cases	Controls	OR (95%CI)	P value
A-1377	A-670	0.032	0.022	1.490 (0.615 - 3.614)	0.37
A-1377	G-670	0.180	0.169	1.081 (0.745 - 1.568)	0.68
G-1377	A-670	0.613	0.661	0.812 (0.605 - 1.089)	0.16
G-1377	G-670	0.175	0.148	1.217 (0.828 - 1.789)	0.31
D' (r^2), 0.763 (0.285)			0.831 (0.351)		

(5). In the present study, the -1377G-670A had a higher frequency in controls rather than in patients, but the difference was not significant. In the United Kingdom, and in patients with acute myeloid leukemia, this was the same about -1377G-670A, and there was a significant higher frequency among patients in comparison with controls for -1377A-670A (15). In Chinese patients with breast cancer, *fas*-1377A/-670A had a significant higher frequency among

controls in comparison with patients; however, the same study showed an increased risk of breast cancer in subjects with the *fas*-1377G/-670A (3). In addition to SNPs analysis, we investigated the correlation between risk of breast cancer and combined genotypes in the *fas* promoter. Results of the present study showed that the -1377GG-670AA had, noticeably, a higher frequency in controls rather than patients. The contrast result was obtained about -1377GA-670AG that was more frequent among patients, but the differences were not significant for both of combinations. To the best of the authors' knowledge, none of the previous studies have demonstrated the association between genotype combination status of 2 most common *fas* promoter polymorphisms and cancer risk to make a comparative interpretation (2, 3, 5, 9). In addition, the genotype frequency in Iranian control subjects was consistent with the results on other diseases in Iran. For example, the frequencies of the fas-1377GG,GA, and AA in our control group were 65.59%, 30.64%, and 3.76%, respectively, which was similar to those found by Mohammadi et al. 72%, 25%, and 3%, respectively. The frequency of the *fas*-1377A allele in our healthy subjects was 19.09%, which was also similar to that reported by Mohammadi et al. (29), 15.5%. The agreement between the data of this study and those of other studies with Iranian subjects suggests that any genotyping bias in the estimation of the variant allele frequencies is not substantial (29). Polymorphisms within promoter may influence transcription factor interaction with DNA sequence (34). The position of -1377G > A/-670A > G is in the promoter of *fas* that may influence protein binding to DNA. It was shown that the reduced *fas* expression level on cells is often correlated with various malignancies, such as breast tumors (5). It was believed that the transcription factors had an important role in balancing transcription and translation genes. It was reported that transcription factors, Sp1, STAT1, (5) ADD1/SREBP1 (35), and P53 (36) are correlated with the *fas* transactivation. Recently, in silico applications were used to analyze the polymorphisms effects in gene functions (37). In the present study, PNI modeler results showed that the *fas* promoter transitions (-1377G > A/-670A > G) altered protein binding pattern in the promoter around SNPs that might also change the interaction of transcription factors, such as Sp1/STAT1 with the *fas* promoter and might result in postulated altered *fas* transcription. To the best of the authors' knowledge, none of the previous studies have investigated the effect of *fas* promoter polymorphisms on the binding pattern of proteins through in silico analysis. The present investigation has some limitations. One of the limitations of this study is the lack of environmental agents that effect determination, such as diet, physical activity, the use of oral contraceptives, which may influence breast cancer risk via gene-environment interactions. Insufficient patient population for further sub-group studies and the lack of family history information of patients could be another limitation of this study. One of the other limitations of this study is the lack of investigation about the effect of -1377G > A-670A > G haplotypes on cancer initiation/progression in molecular level. If the results of this study are consistent with the results of large studies and supported by in vitro investigations, they can be useful in the determination of relative risk of breast tumors development in northwest of Iran.

4.1. Conclusions

In conclusion, the results of this study did not show any significant associations between 2 polymorphisms of *fas* promoter and breast cancer risk. However, further investigations are required to validate these conclusions across different populations. Moreover, PNI modeler program showed that the binding pattern of proteins to the promoter of the *fas* gene changes due to the presence of -1377G > A, -670A > G polymorphisms in the promoter.

Acknowledgments

The authors thank Imam Reza/Noor-E-Nejat Hospitals staff for their cooperation in sample and clinicpathological data collection procedure. Likewise, authors appreciate all patients and healthy persons for their cooperation.

Footnotes

Authors' Contribution: Study concept and design, All authors; acquisition of data, Narges Dastmalchi, Reza Safaralizadeh, Nasser Pouladi; analysis and interpretation of data, Narges Dastmalchi, Nasser Pouladi; drafting the manuscript, All authors; critical revision of the manuscript for important intellectual content, Mohammad Ali Hosseinpour Feizi; statistical analysis, Narges Dastmalchi, Nasser Pouladi; administrative, technical, and material support, Mohammad Ali Hosseinpour Feizi, Narges Dastmalchi; study supervision, Mohammad Ali Hosseinpour Feizi, Reza Safaralizadeh, Nasser Pouladi.

Conflict of Interests: None declared.

Financial Disclosure: The authors declare that they have no conflict of interest.

Funding/Support: None declared.

References

1. Babu GR, Samari G, Cohen SP, Mahapatra T, Wahbe RM, Mermash S, et al. Breast cancer screening among females in Iran and recommendations for improved practice: a review. *Asian Pac J Cancer Prev.* 2011;**12**(7):1647–55. [PubMed: 22126539].

2. Hashemi M, Fazaeli A, Ghavami S, Eskandari-Nasab E, Arbabi F, Mashhadi MA, et al. Functional polymorphisms of FAS and FASL gene and risk of breast cancer - pilot study of 134 cases. *PLoS One.* 2013;**8**(1):53075. doi: 10.1371/journal.pone.0053075. [PubMed: 23326385].
3. Xu Y, Deng Q, He B, Pan Y, Li R, Gao T, et al. The diplotype Fas -1377A/-670G as a genetic marker to predict a lower risk of breast cancer in Chinese women. *Tumour Biol.* 2014;**35**(9):9147–61. doi: 10.1007/s13277-014-2175-7. [PubMed: 24916571].
4. Stecklein SR, Jensen RA, Pal A. Genetic and epigenetic signatures of breast cancer subtypes. *Front Biosci (Elite Ed).* 2012;**4**:934–49. [PubMed: 22201926].
5. Zhang B, Sun T, Xue L, Han X, Zhang B, Lu N, et al. Functional polymorphisms in FAS and FASL contribute to increased apoptosis of tumor infiltration lymphocytes and risk of breast cancer. *Carcinogenesis.* 2007;**28**(5):1067–73. doi: 10.1093/carcin/bgl250. [PubMed: 17183065].
6. Nichols KE, Malkin D, Garber JE, Fraumeni JJ, Li FP. Germ-line p53 mutations predispose to a wide spectrum of early-onset cancers. *Cancer Epidemiol Biomarkers Prev.* 2001;**10**(2):83–7. [PubMed: 11219776].
7. Dalan AB, Timirci-Kahraman O, Turan S, Kafadar AM, Yaylim I, Ergen A, et al. Association between FAS and FASL genetic variants and risk of primary brain tumor. *Int J Neurosci.* 2014;**124**(6):443–9. doi: 10.3109/00207454.2013.850083. [PubMed: 24094130].
8. Zhang Z, Wang LE, Sturgis EM, El-Naggar AK, Hong WK, Amos CI, et al. Polymorphisms of FAS and FAS ligand genes involved in the death pathway and risk and progression of squamous cell carcinoma of the head and neck. *Clin Cancer Res.* 2006;**12**(18):5596–602. doi: 10.1158/1078-0432.CCR-05-1739. [PubMed: 17000697].
9. Zhang Z, Xue H, Gong W, Wang M, Yuan L, Han S, et al. FAS promoter polymorphisms and cancer risk: a meta-analysis based on 34 case-control studies. *Carcinogenesis.* 2009;**30**(3):487–93. doi: 10.1093/carcin/bgp016. [PubMed: 19168581].
10. Kim R, Emi M, Tanabe K, Uchida Y, Toge T. The role of Fas ligand and transforming growth factor beta in tumor progression: molecular mechanisms of immune privilege via Fas-mediated apoptosis and potential targets for cancer therapy. *Cancer.* 2004;**100**(11):2281–91. doi: 10.1002/cncr.20270. [PubMed: 15160330].
11. Zhou RM, Wang N, Chen ZF, Duan YN, Sun DL, Li Y. Polymorphisms in promoter region of FAS and FASL gene and risk of cardia gastric adenocarcinoma. *J Gastroenterol Hepatol.* 2010;**25**(3):555–61. doi: 10.1111/j.1440-1746.2009.06116.x. [PubMed: 20074157].
12. Wang M, Wu D, Tan M, Gong W, Xue H, Shen H, et al. FAS and FAS ligand polymorphisms in the promoter regions and risk of gastric cancer in Southern China. *Biochem Genet.* 2009;**47**(7-8):559–68. doi: 10.1007/s10528-009-9264-0. [PubMed: 19565204].
13. Sung WW, Wang YC, Cheng YW, Lee MC, Yeh KT, Wang L, et al. A polymorphic -844T/C in FasL promoter predicts survival and relapse in non-small cell lung cancer. *Clin Cancer Res.* 2011;**17**(18):5991–9. doi: 10.1158/1078-0432.CCR-11-0227. [PubMed: 21807637].
14. Valibeigi B, Amirghofran Z, Golmoghaddam H, Hajihosseini R, Kamazani FM. Fas gene variants in childhood acute lymphoblastic leukemia and association with prognosis. *Pathol Oncol Res.* 2014;**20**(2):367–74. doi: 10.1007/s12253-013-9705-2. [PubMed: 24218069].
15. Sibley K, Rollinson S, Allan JM, Smith AG, Law GR, Roddam PL, et al. Functional FAS promoter polymorphisms are associated with increased risk of acute myeloid leukemia. *Cancer Res.* 2003;**63**(15):4327–30. [PubMed: 12907599].
16. Crew KD, Gammon MD, Terry MB, Zhang FF, Agrawal M, Eng SM, et al. Genetic polymorphisms in the apoptosis-associated genes FAS and FASL and breast cancer risk. *Carcinogenesis.* 2007;**28**(12):2548–51. doi: 10.1093/carcin/bgm211. [PubMed: 17962219].
17. Bartlett JMS. Ovarian cancer: Methods and protocols. 1st ed. New Jersey: Springer Press; 2000.
18. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. Breast: AJCC cancer staging manual. 7th ed. New York: Springer, New York Press; 2010.
19. Li X, Ding X, Chu B, Ding G, Gu S, Qian L, et al. Molecular authentication of Alisma orientale by PCR-RFLP and ARMS. *Planta Med.* 2007;**73**(1):67–70. doi: 10.1055/s-2006-951746. [PubMed: 17109255].
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**(13):1030–6. [PubMed: 15240787].
21. Dufournet C, Uzan C, Fauvet R, Cortez A, Siffroi JP, Darai E. Expression of apoptosis-related proteins in peritoneal, ovarian and colorectal endometriosis. *J Reprod Immunol.* 2006;**70**(1-2):151–62. doi: 10.1016/j.jri.2005.11.003. [PubMed: 16378643].
22. Farre L, Bittencourt AL, Silva-Santos G, Almeida A, Silva AC, Decanine D, et al. Fas 670 promoter polymorphism is associated to susceptibility, clinical presentation, and survival in adult T cell leukemia. *J Leukoc Biol.* 2008;**83**(1):220–2. doi: 10.1189/jlb.0407198. [PubMed: 17962369].
23. Li C, Wu W, Liu J, Qian L, Li A, Yang K, 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**(4):245–51. doi: 10.1097/01.fpc.0000194425.58511.a7. [PubMed: 16538171].
24. Park JY, Lee WK, Jung DK, Choi JE, Park TI, Lee EB, et al. Polymorphisms in the FAS and FASL genes and survival of early stage non-small cell lung cancer. *Clin Cancer Res.* 2009;**15**(5):1794–800. doi: 10.1158/1078-0432.CCR-08-1770. [PubMed: 19240174].
25. Gaddipati H, Herlyn M. Association of polymorphisms of FAS-1377, FAS-670, and FASL-844 genes with risk factors for cutaneous malignant melanoma. *Cancer J.* 2007;**13**(4):226–8. doi: 10.1097/PPO.0b013e31813fffdd. [PubMed: 17762755].
26. Yildir S, Sezgin M, Barlas IO, Turkoz G, Ankarali HC, Sahin G, et al. Relation of the Fas and FasL gene polymorphisms with susceptibility to and severity of rheumatoid arthritis. *Rheumatol Int.* 2013;**33**(10):2637–45. doi: 10.1007/s00296-013-2793-1. [PubMed: 23749041].
27. Lima L, Morais A, Lobo F, Calais-da-Silva FM, Calais-da-Silva FE, Medeiros R. Association between FAS polymorphism and prostate cancer development. *Prostate Cancer Prostatic Dis.* 2008;**11**(1):94–8. doi: 10.1038/sj.pcan.4501002. [PubMed: 17667965].
28. Xu Y, He B, Li R, Pan Y, Gao T, Deng Q, et al. Association of the polymorphisms in the Fas/FasL promoter regions with cancer susceptibility: a systematic review and meta-analysis of 52 studies. *PLoS One.* 2014;**9**(3):90090. doi: 10.1371/journal.pone.0090090. [PubMed: 24598538].
29. Mohammadi A, Tajik N, Shah-Hosseini A, Alavian SM, Sharifi Z, Jarahi L. FAS and FAS-Ligand Promoter Polymorphisms in Hepatitis B Virus Infection. *Hepat Mon.* 2015;**15**(10):26490. doi: 10.5812/hepatmon.26490. [PubMed: 26587033].
30. Han W, Zhou Y, Zhong R, Wu C, Song R, Liu L, et al. Functional polymorphisms in FAS/FASL system increase the risk of neuroblastoma in Chinese population. *PLoS One.* 2013;**8**(8):71656. doi: 10.1371/journal.pone.0071656. [PubMed: 23951214].
31. Zeng J, Fang Y, Li P. FAS-1377 A/G polymorphism in breast cancer: a meta-analysis. *Tumour Biol.* 2014;**35**(3):2575–81. doi: 10.1007/s13277-013-1339-1. [PubMed: 24185967].
32. Li K, Li W, Zou H, Zhao L. Association between FAS 1377G>A polymorphism and breast cancer susceptibility: a meta-analysis. *Tumour Biol.* 2014;**35**(1):351–6. doi: 10.1007/s13277-013-1048-9. [PubMed: 23979975].
33. Verim L, Timirci-Kahraman O, Akbulut H, Akbas A, Ozturk T, Turan S, et al. Functional genetic variants in apoptosis-associated FAS and FASL genes and risk of bladder cancer in a Turkish population. *In Vivo.* 2014;**28**(3):397–402. [PubMed: 24815844].
34. Nadeau JH. Single nucleotide polymorphisms: tackling complexity. *Nature.* 2002;**420**(6915):517–8. doi: 10.1038/420517a. [PubMed: 12466848].
35. Kim JB, Sarraf P, Wright M, Yao KM, Mueller E, Solanes G, et al. Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. *J Clin Invest.* 1998;**101**(1):1–9.

doi: 10.1172/JCI1411. [PubMed: 9421459].
36. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong S. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. *Cell Death Dis.* 2011;**2**:148. doi: 10.1038/cddis.2011.33. [PubMed: 21509038].
37. Aftabi Y, Colagar AH, Mehrnejad F. An in silico approach to investigate the source of the controversial interpretations about the phenotypic results of the human AhR-gene G1661A polymorphism. *J Theor Biol.* 2016;**393**:1–15. doi: 10.1016/j.jtbi.2016.01.001. [PubMed: 26776670].