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Strong Linkage Disequilibrium Between -670 A>G and -1377 G>A Polymorphisms in FAS Gene in Patients with Breast Cancer and Controls

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ABSTRACT

Breast cancer is the second cause of mortality in women. The etiology of breast cancer is multi factorial. Genetic factors play an important role in the etimology of breast cancer. The FAS gene, has a critical role in the tumor growth and metastasis. Gene polymorphisms including -1377 G>A and -670 A/G in FAS gene have shown to change the transcription activities of this gene. The FAS genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 115 breast cancer patients, 115 healthy controls, all female and selected from city of Mashhad in north-eastern part of Iran. BstuI and ScrfI were used as endonuclease enzymes to detect -1377G/A and -670A/G gene polymorphisms, respectively. GG, GA and AA genotype frequencies for FAS -1377 polymorphism in patients were 30.4%, 49.6% and 20%, whereas in healthy controls were 26.1%, 50.4% and 23.5%, respectively and there was no significant difference between case and controls (p=0.7).Additionally, genotype frequencies of FAS -670 AA, AG and GG in patient groups were 52.2%, 39.1% and 8.7% respectively . The result of genotype frequency in controls for FAS -670 AA, AG and GG was 47%, 41% and 10.4%, which Showed insignificant difference as compared with patients genotypes (p=0.78). These results showed that FAS−1377G/A and FAS -670 A/G gene polymorphisms had a strong linkage disequilibrium with p value <0.001. These results indicated the lack of association between FAS−1377G>A and FAS -670 A/G gene polymorphisms and risk of breast cancer. Moreover, a significant linkage disequilibrium was found betweenFAS−1377G/A and FAS -670 A/G gene polymorphisms in case and control groups.

Keywords: Breast Cancer, -FAS−1377G>A, FAS -670 A/G,Linkage Disequilibrium, Gene Polymorphism

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Breast cancer is developed from the breast tumors. There are more than 18 sub-types of this cancer. This type of cancer is the second cause of mortality in women (1,2). The etiology of breast cancer is multi factorial. Genetic factors are interfered in the etiology of breast cancer. The FAS gene, has a critical role in the tumor growth and metastasis (2). Two polymorphisms have been identified in the FAS promoter region one in the silencer region, G to A substitution at nucleotide position −670 (rs1800682) (3).

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These polymorphisms have shown to change the transcription activities of FAS gene[4].In the present study, We aimed to determine the linkage disequilibrium between -670 A>G and -1377 G>A polymorphisms in FAS gene in patients with breast cancer and controls.

Materials and Methods

We carried out a case-control genetic study in a population selected from Omid Cancer Hospital of Mashhad in north - eastern part of Iran during the period from February 2013 - October 2014. This study involved a group of 115 female patients with histologically-confirmed diagnosis of Breast cancer and 115 healthy controls with negative medical history. DNA was extracted by DNA extraction kit.

Table1. Reaction setup

	FAS -1377 G/A lµVolume – based	FAS – 670A/G lµVolume – based
Genomic DNA(100ng)	1	1
dNTP(0.2µm)	0.4	0.4
PCR buffer(10x)	2	2
MgCl2 (25µm)	0.6	0.6
Reverse Primer(10pmol)	0.8	0.8
Forward Primer(10pmol)	0.8	0.8
Taq DNA polymerase(5 U/µL)	1	1
Deionised H2O	13.4	13.4
Total	20	20

Spectrophotometry and 1 % agarose gel electrophoresis methods were used to determine quality of the extracted DNA.

The primer sequences were as follows:

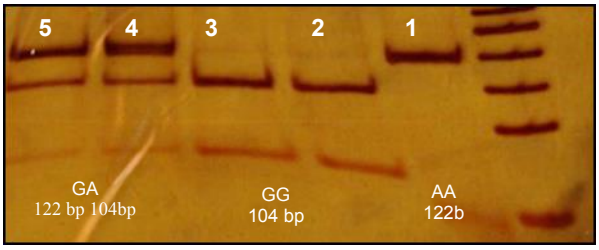
FAS -1377 G/A
F 5′-TGTGTGCACAAGGCTGGCGC -3'
R 5′-TGCATCTGTCACTGCACTTACCACCA- 3
FAS -670 A/G
F 5′- ATAGCTGGGGCTATGCGATT-3'
R 5′-CATTTGACTGGGCTGTCCAT-3'

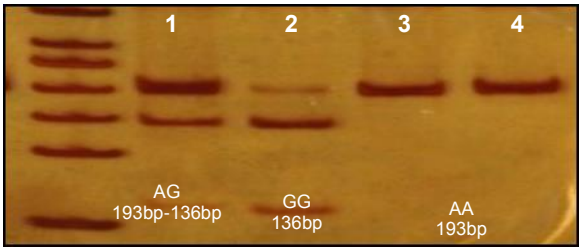
These target sequences were amplified according to the recipe in Table1. PCR amplifications were conducted in Personal Cycler™ amplificator (Biometra, Germany). Thermal cycling conditions were as follows Table 2.

Table2. Thermocycling conditions for PCR

	1377-		670-	
	Temperature °C	Time	Tempera-ture °C	Time
Initial denatura-tion for1cycle	94	2 min	94	2 min
Denaturation	94	30 s	94	30 s
Annealing	69	30 s	62	30 s
Extension	72	45 s	72	45 s
Go to step 2 for35cycles				
final extension	72	min 7	72	min 7

4 µL of PCR products were digested overnight at 37°C in a 10 µL reaction volume containing 1 units of BstuI and ScrfI (Fermentas, Germany) were recruited as endonuclease enzymes to detect -1377G/A and -670A/G gene polymorphisms, respectively. After overnight , the digestion solutions were analyzed on 17% acrylamide gel and then visualized with silver-nitrate staining. (Figure 1),(Figure 2).





Restriction digestion analysis of PCR products with and on 17% acrylamide gel.

(Fig.1) FAS-1377G/A Lane1: AA homozygote with 122 bp ; Lane 2,3: GG homozygote with 104bp; Lane4,5: GA heterozygote genotype with 122bp and 104bp .Size marker of DNA (50bp ladder).

(Fig.2) FAS-670 A/G Lane1: AG heterozygote genotype with 193bp and 136bp, Lane 2: 136 bp GG homozygote and lane3,4:193bp AA homozygote respectively; Size marker of DNA (100bp ladder).

Statistical analysis

The statistical analysis of the data was performed by using the SPSS 18.0 software. Genotypes and alleles were compared between groups by use of $\chi 2$ test. The strength of the association between the polymorphisms and cancer risk was measured by odds ratios (ORs) with 95% confidence intervals (CIs). The allele frequencies of 2 polymorphisms in case and control groups followed Hardy-Weinberg's law of Equilibrium ($P > 0.05$) .

Results

The frequencies of the two alleles in breast cancer patients were compared with the frequencies in the healthy control subjects. In the same order , GG, GA and AA genotype frequencies for FAS -1377 polymorphism in patients were 30.4%, 49.6% and 20%, whereas in control groups were 26.1%, 50.4% and 23.5%, and there was no significant difference between case and control groups. Furthermore, genotype frequencies of FAS -670 AA, AG and GG in patients were 52.2%, 39.1% and 8.7% respectively. The result of genotype frequencies in control groups for FAS -670 AA, AG and GG were 47%, 41% and 10.4%, which Showed insignificant difference as compared with patients genotypes .These results showed that FAS−1377G/A and FAS -670 A/G gene polymorphisms had a strong linkage disequilibrium with (p value <0.001).

Discussion

Several polymorphism sites have been identified to influence the development of breast cancer (2,7). Death receptor activation triggers extrinsic apoptotic pathways, which in some cell types may be enough to carry apoptosis (11-13).

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Several previous researches concentrated on the association between different gene polymorphisms within cell death pathways and the risk of developing malignancies and developing cancers. FAS polymorphisms may play different, context-dependent roles in cancer (14,15) Crew et al., found no association between breast cancer and FAS -1377 G/A, FAS-670 G/A polymorphisms (16). On the other hand, Zhang et al., reported an important association between FAS 1377G/A gene polymorphism and risk of breast cancer, but they reported no association between FAS -670 G/A and breast cancer risk (17).

Hashemi et al., mentioned that the FAS-1377 G/A did not affect the risk of breast cancer, while FAS -670 G/A gene polymorphism was risk factor[4]. Krippl et al., in a study of 500 breast cancer patients and 500 controls in a Caucasian population in Austria, found a significant association between FAS-1377G/A and increased risk of breast cancer, but they reported no associations with FAS -670G/A (18).

Our study indicates the lack of association between FAS−1377G>A and FAS -670 A/G gene polymorphisms and risk of breast cancer. Also, a main linkage disequilibrium is found betweenFAS−1377G/A and FAS -670 A/G gene polymorphisms in case and control groups.

One of the limitation in our study is small sample size.

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Conclusions

While there have been current discussions on the influence of various polymorphisms within the signaling molecules relevant to cell death pathways, and cancer formation , this work has proved its worth. Also , we suggest to study the other candidate gene polymorphism which may be beneficial for identifying the factors involved in breast cancer.