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FEN1 -69G>A and +4150G>T polymorphisms and breast cancer risk

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Abstract. Flap endonuclease 1 (FEN1), a DNA repair protein, is important in preventing carcinogenesis. Two functional germ line variants -69G>A (rs174538) and +4150G>T (rs4246215) in the *FEN1* gene have been associated with risk of various types of cancer. The aim of the present study was to evaluate the possible impact of *FEN1* polymorphisms on risk of breast cancer (BC) in a sample of Iranian subjects. The *FEN1* -69G>A and +4150G>T polymorphisms were analyzed in a case-control study that included 266 BC patients and 225 healthy females. Polymerase chain reaction-restriction fragment length polymorphism analysis was used to genotype the variants. The findings demonstrated that the *FEN1* -69G>A and +4150G>T polymorphisms were not associated with BC risk in co-dominant, dominant and recessive inheritance models. The findings indicated that GG/GT, GA/GG and GA/TT genotypes significantly decreased the risk of BC when compared with -69GG/+4150GG. Furthermore, haplotype analysis indicated that -69G/+4150T as well as -69A/+4150G significantly decreased the risk of BC compared with -69G/+4150G. Thus, these findings demonstrated that haplotypes of *FEN1* -69G>A and +4150G>T polymorphisms decreased the risk of BC in an Iranian population. Further studies with larger sample sizes and different ethnicities are required to validate the present findings.

Introduction

Breast cancer (BC) is the most common type of cancer and the second leading cause of cancer-associated mortality among women globally (1). It is documented as a significant worldwide health care problem affecting ~1 million women annually (1-3). BC is also recognized as one of the most frequent cancer types among Iranian women, comprising 21.4% of cases of female cancer (4). Genetic and environmental factors are involved in BC pathogenesis; however, its exact etiology is complicated and not clearly identified (5). A previous study demonstrated that genetic factors are important in the development of BC in populations in the southeast of Iran (6-11).

Somatic mutations and variants in flap endonuclease 1 (FEN1), an essential enzyme involved in DNA replication and repair, lead to functional defects of the FEN1 protein and a susceptibility to cancer (12). *FEN1* is an important tumor suppressor gene. It is a structure-specific nuclease that is involved in 5'-flap removal during long-patch base-excision repair and the maturation of Okazaki fragments in DNA replication (13,14). Furthermore, FEN1 is also characterized as a 5' exonuclease (15) and a gap-dependent endonuclease (16), which can be stimulated to promote apoptotic DNA fragmentation in response to apoptotic stimuli. Due to the critical role of FEN1 in DNA repair and various other DNA metabolic pathways, it has been proposed that FEN1 serves as a key enzyme in maintaining genomic stability and protecting against carcinogenesis (15).

The *FEN1* gene is located on the long arm of chromosome 11 (11q12.2). The gene is polymorphic and two functional variants, -69G>A (rs174538) and +4150G>T (rs4246215), have become points of interest. Previous studies have proposed that the two variants in *FEN1* may function as biomarkers for BC, glioma, hepatocellular carcinoma, and esophageal, gastric, colorectal and lung cancer, as well as childhood leukemia (12,17-20).

To the best of our knowledge there are no studies regarding the association between FEN1 variants and BC in the Iranian population. Therefore, the present study aimed to establish the impact of -69G>A (rs174538) and +4150G>T (rs4246215) gene polymorphisms on BC risk in an Iranian population.

Patients and methods

Patients. This population-based case-control study was performed on 266 females, unrelated pathologically

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confirmed BC patients who were referred to the Ali-ibn Abi Talib Hospital (Zahedan, Iran) from May 2010 until June 2014 and 225 age-matched population-based healthy women who participated in a screening project for metabolic syndrome with no history of any type of cancer, and were not related to the BC patients. The enrollment procedure and study design has been described previously (7,21-24).

Ethical approval for subject and patient recruitment was obtained from the local Ethics Committee of Zahedan University of Medical Sciences (Zahedan, Iran), and informed consent was obtained from all patients and healthy individuals. Blood samples (2 ml) were collected from patients and healthy controls, stored in EDTA-containing tubes and the DNA was extracted according to the salting-out method, as described previously (25). The quality of the purified DNA was assessed by electrophoresis on 1% agarose gel (100 V for 20 min), quantitated spectrophotometrically by measuring the absorbance at 260 nm (Lambda 25; PerkinElmer, Inc., Waltham, MA, USA), and stored at -20˚C until further use.

Genotyping. Genotyping of the *FEN1* -69G>A (rs174538) polymorphism was performed using polymerase chain reaction (PCR)-restriction fragment length polymorphism, as described previously (19). Briefly, for -69G>A genotyping the forward and reverse primers were 5'-GGAGGTTCC AGGAGCGTCTA-3' and 5'-TTCTCCACCGCTTGTCCC-3', respectively. PCR reaction solution (0.20 ml) included 1 μl genomic DNA (~100 ng/ml), 1 μl forward and reverse primers and 10 μl 2X Prime Taq Premix (Genet Bio, Inc., Daejeon, Korea) and 7 μl ddH_2O. The amplification procedure consisted of an initial denaturing step for 5 min at 95˚C followed by 30 cycles for 30 min at 95˚C, 30 min at 62˚C, and 30 min at 72˚C, and a final extension step for 5 min at 72˚C. PCR product (10 μl) was digested using *Sal*I restriction enzyme (New England Biolabs, Inc., Ipswich, MA, USA). The PCR products were visualized on 2.5% agarose gel containing 0.5 μg/ml of ethidium bromide in a UV transilluminator (DigiDoc H101) and photographed. The A allele was digested resulting in 171 and 150-bp products, while the undigested G allele resulted in a 321-bp product.

For *FEN1* and +4150G>T (rs4246215), the forward and reverse primers were 5'-CCCAGAATATTTGCCGTCTTGT-3' and 5'-CAGCCAGTAATCAGTCACAAACAC-3', respectively. PCR was performed using 2X Prime Taq Premix (Genet Bio, Inc.). The amplification procedure consisted of an initial denaturing step for 5 min at 95˚C followed by 30 cycles for 30 min at 95˚C, 30 min at 64˚C, and 30 min at 72˚C, and a final extension step for 5 min at 72˚C. PCR product (10 μl) was digested using Alw26I restriction enzyme (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The PCR products were visualized on 2.5% agarose gel containing 0.5 μg/ml of ethidium bromide in a UV transilluminator (DigiDoc H101) and photographed. The PCR product was 523-bp; the G allele was digested to give 289-bp and 234-bp products, while the T allele was undigested.

Statistical analysis. All statistical analyses were performed using SPSS 20 software (IBM SPSS, Armonk, NY, USA). The differences between the variables were assessed using the χ^2 test or the independent sample t-test. The Hardy-Weinberg equilibrium (HWE) was assessed in cases and controls by χ^2 test. The associations between genotypes and BC were calculated by computing the odds ratio and 95% confidence intervals from logistic regression analyses. P<0.05 was considered to indicate statistically significant differences.

Results

Patient characteristics. The study groups included 266 pathologically confirmed BC patients (mean age, 48.9±11.2 years) and 225 healthy women (mean age, 50.0±13.0 years). There was no significant difference between the groups regarding age (P=0.306).

Associations between the variants and BC risk. The frequency distribution of the *FEN1* polymorphisms in the BC cases and control subjects are summarized in Table I. The results indicate that the *FEN1* -69G>A and +4150G>T polymorphisms were not associated with the risk of BC in any inheritance model that was evaluated. The interaction of the two variants of the *FEN1* gene were determined and the results demonstrated that GG/GT, GA/GG, and GA/TT genotypes significantly decreased the risk of BC (P=0.012, P<0.001 and P=0.002, respectively) compared with -69GG/+4150GG genotype (Table II).

Haplotype analysis indicated that -69G/+4150T as well as -69A/+4150G significantly decreased (P<0.0001) the risk of BC compared with -69G/+4150G (Table III).

The *FEN1* -69G>A and +4150G>T genotypes in the two controls and cases were at HWE (P>0.05).

Associations between the variant and clinicopathological characteristics. In Table IV, the association between *FEN1* variants and clinicopathologic parameters, including age, pathological type, tumor stage (according to the American Joint Committee on Cancer and the International Union for Cancer Control classification system for tumor, nodes, and metastases), tumor grade (according to the Elston-Ellis grading system), estrogen and progesterone receptors and human growth factor receptor 2 (HER2) status are presented. The -69G>A (rs174538) was identified to be associated with the pathologic type and HER2 status of tumor (P<0.05), and the +4150G>T (rs4246215) variant was associated with the PgR and HER2 status (P<0.05).

Discussion

It is hypothesized that DNA repair genes are involved in maintenance of genomic stability and prevention of carcinogenesis. Among these genes, *FEN1* is involved in base excision repair and DNA replication. Previous studies have focused on two variants, the -69G>A polymorphism (located in the promoter region) and +4150 G>T (located in the 3'-untranslated region) of the *FEN1* gene, and the risk of various types of cancer (12,17-19). These variants have been revealed to be functional and affect the expression level of FEN1 (19). In the present study the impact of -69G>A and +4150 G>T functional variants on BC risk was investigated in an Iranian population. The findings proposed that the *FEN1* -69G>A and +4150G>T polymorphisms were not associated with the risk of BC in this population. GG/GT, GA/GG and GA/TT genotypes

Table I. Association between the *FEN1* -69 G>A (rs174538) and +4150 G>T (rs4246215) gene polymorphisms, and BC risk.

FEN1 polymorphisms	BC, n (%)	Control, n (%)	Odds ratio (95% CI)	P-value
rs174538 G>A				
Co-dominant				
GG	165 (62.0)	125 (55.6)	1.00	-
GA	87 (32.7)	92 (40.8)	0.72 (0.49-1.04)	0.087
AA	14 (5.3)	8 (3.6)	1.33 (0.54-3.26)	0.656
Dominant				
GG	165 (62.0)	125 (55.6)	1.00	-
GA+AA	101 (38.0)	100 (43.4)	0.77 (0.53-1.10)	0.167
Recessive				
GG+GA	242 (94.7)	217 (96.4)	1.00	-
AA	14 (5.3)	8 (3.6)	1.57 (0.65-3.81)	0.384
Allele				
G	417 (78.4)	342 (76.0)	1.00	-
A	115 (21.6)	108 (24.0)	0.87 (0.66-1.18)	0.401
rs4246215 G>T				
Co-dominant				
GG	138 (51.9)	104 (46.2)	1.00	-
GT	111 (41.7)	97 (43.1)	0.86 (0.59-1.25)	0.448
TT	17 (6.4)	24 (10.7)	0.53 (0.27-1.05)	0.089
Dominant				
GG	138 (51.9)	104 (46.2)	1.00	-
GT+TT	128 (48.1)	121 (56.0)	0.80 (0.56-1.14)	0.239
Recessive				
GG+GT	249 (93.6)	201 (89.3)	1.00	-
TT	17 (6.4)	24 (10.7)	0.57 (0.30-1.09)	0.102
Allele				
G	387 (72.7)	305 (67.8)	1.00	-
T	145 (27.3)	145 (32.2)	0.79 (0.60-1.04)	0.092

FEN1, flap endonuclease 1; BC, breast cancer; CI, confidence interval.

Table II. Interaction of *FEN1* -69G>A (rs174538) and +4150G>T (rs4246215) gene polymorphisms on BC risk.

-69G>A (rs174538)	+4150G>T (rs4246215)	BC, n (%)	Control, n (%)	Odds ratio (95% CI)	P-value
GG	GG	132 (49.6)	77 (34.2)	1.00	-
GG	GT	33 (12.4)	39 (17.3)	0.49 (0.29-0.85)	0.012
GA	GT	78 (29.3)	55 (24.4)	0.83 (0.53-1.29)	0.426
AA	TT	14 (5.3)	3 (1.3)	2.72 (0.76-9.78)	0.185
GA	GG	6 (2.3)	25 (11.1)	0.14 (0.06-0.36)	<0.001
GA	TT	3 (1.1)	12 (5.3)	0.15 (0.04-0.53)	0.002
AA	GG	0 (0.0)	2 (0.9)	-	-
GG	TT	0 (0.0)	9 (4.0)	-	-
AA	GT	0 (0.0)	3 (1.3)	-	-

FEN1, flap endonuclease 1; BC, breast cancer; CI, confidence interval.

significantly decreased the risk of BC when compared with the -69GG/+4150GG genotype. Furthermore, haplotype analysis demonstrated that -69G/+4150T and -69A/+4150G significantly reduced the risk of BC compared with -69G/+4150G.

Table III. Haplotype association of -69G>A (rs174538) and +4150G>T (rs4246215) variants with BC risk.

-69G>A (rs174538)	+4150G>T (rs4246215)	BC (frequency)	Control (frequency)	Odds ratio (95% CI)	P-value
G	G	0.7153	0.5888	1.00	-
A	T	0.2041	0.1510	0.99 (0.69-1.41)	0.940
G	T	0.0685	0.1712	0.34 (0.22-0.54)	<0.0001
A	G	0.0121	0.0890	0.12 (0.05-0.29)	<0.0001

BC, breast cancer; CI, confidence interval.

Table IV. Association between *FEN1* -69G>A (rs174538) and +4150G>T (rs4246215) polymorphisms and clinicopathological characteristics.

	-69G>A (rs174538)				+4150G>T (rs4246215)			
Variables	GG	GA	AA	P-value	GG	GT	TT	P-value
Age (years)				0.757				0.896
≤50	88	52	8		75	63	10	
>50	71	35	5		58	47	6	
Pathological type				0.014				0.236
Ductal	116	50	9		95	69	11	
Other	34	34	2		31	36	6	
Tumor size (cm)				0.579				0.379
≤2	50	32	6		40	41	7	
>2	103	52	8		89	64	10	
TNM stage				0.202				0.338
I	25	18	1		21	22	1	
II	55	36	8		49	41	9	
III	43	25	2		33	33	4	
IV	31	6	3		27	10	3	
Tumor grade				0.588				0.380
I	32	12	2		26	17	3	
II	79	51	8		64	65	9	
III	32	11	2		29	14	2	
IV	1	0	0		0	1	0	
Estrogen receptor status				0.298				0.821
Positive	91	54	9		82	60	12	
Negative	57	21	5		43	35	5	
PgR status				0.508				0.029
Positive	96	43	8		87	50	10	
Negative	51	32	5		37	45	6	
HER2 status				0.012				0.002
Positive	69	54	5		56	67	5	
Negative	84	31	9		73	39	12	

Tumor stage was established according to the American Joint Committee on Cancer and the International Union for Cancer Control classification system for TNM. The tumor grade was established according to the Elston-Ellis grading system. *FEN1*, flap endonuclease 1; TNM, tumor, node, metastasis; HER2, human growth factor receptor 2; PgR, progesterone receptor.

Lv *et al* (18) examined the possible association between the *FEN1* -69G>A (rs174538) and 4150G>T (rs4246215) polymorphisms and risk of BC in a Chinese population. *FEN1* -69G and +4150G alleles, which are correlated to significantly decreased *FEN1* mRNA expression levels in normal breast tissues, were associated with increased BC risk when

compared with -69A and 4150T alleles in two independent case-control sets (18).

A genome-wide association study performed by Zhang *et al* (26) indicated that rs4246215 (4150G>A), variant of the *FEN1* gene, was associated with colorectal cancer in an East Asian population.

Yang *et al* (19) reported that *FEN1* -69GG or +4150GG carriers were associated with a significantly increased risk for developing lung cancer in comparison with the -69AA or 4150TT carriers. Furthermore, Liu *et al* (12) investigated the association between -69G>A (rs174538) and 4150G>T (rs4246215) polymorphisms and gastrointestinal cancer risk in two independent case-control cohorts. The allelic frequencies for the -69A and +4150T were 0.364 and 0.360 for healthy controls in the Jinan cohort and 0.458 and 0.457 for control subjects in the Huaian cohort. The distribution of allelic frequencies of the two variants was significantly different between the Jinan and Huaian Chinese populations (P<0.0001). The authors found that the *FEN1* -69GG and the +4150GG genotypes significantly increased the risk of hepatocellular carcinoma, and esophageal and gastric cancer in the two populations. Although, no significant association between *FEN1* -69G>A and +4150G>T variants, and colorectal cancer was identified in the studied population. The authors concluded that the *FEN1* -69G and +4150G alleles, which are associated with a significantly decreased *FEN1* mRNA expression level in normal gastrointestinal tissues, are associated with increased gastrointestinal cancer risk (12).

A meta-analysis performed by Gao *et al* (27) proposed that *FEN1* -69G>A and +4150G>T polymorphisms may be associated with cancer susceptibility in a Chinese population. The findings revealed that subjects with -69A or +4150T alleles of the *FEN1* gene were associated with a decreased risk of cancer. A recent meta-analysis designated that FEN1 rs174538 and rs4246215 polymorphisms may contribute to an increased risk of cancer (28). The findings indicated that the GG or GA genotype increased the risk of cancer when compared to the rs174538 AA genotype. For rs4246215, the GG or GT genotype was significantly associated with an increased cancer risk when compared with the TT genotype.

Pei *et al* (20) investigated the impact of two functional polymorphisms of *FEN1* on the risk of childhood acute lymphoblastic leukemia (ALL). It was found that the AG and AA genotypes of the rs174538 variant, but not the rs4246215 variant, significantly decreased the risk of childhood ALL. In addition, Chen *et al* (17) found that the *FEN1* -69GG or +4150GG genotype significantly increased the risk of glioma when compared with the -69AA or +4150TT genotype.

In conclusion, the findings of the present study support an association between *FEN1* haplotypes and the risk of BC in an Iranian population from the southeast of Iran. However, a larger sample size and subjects from different ethnicities are required to confirm the current findings.

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