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Association of FGFR2 and TOX3 Genetic Variants With the Risk of Breast Cancer in Iranian Women

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ABSTRACT

Background: Breast cancer is the most common cause of cancer-related death in women worldwide. Novel genetic markers for breast cancer susceptibility have been identified in population-based studies. The aim of this study was to examine the association of two single-nucleotide polymorphisms (SNPs) of *FGFR2* (rs1219648) and *TOX3* (rs8051542) with the risk of breast cancer in Iranian women.

Methods: Breast cancer patients (n = 126) and healthy controls (n = 160) were genotyped for SNPs in *FGFR2* (rs1219648) and *TOX3* (rs8051542) using the tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). Also, immunohistochemical tests for human epidermal growth factor receptor-2, estrogen receptor, and progesterone receptor were carried out on breast tumor tissues.

Results: *TOX3* (rs8051542) CC (OR = 1.24; 95% CI, 0.72-0.214; P < 0.001) and *FGFR2* (rs1219648) GG (OR = 62.0; 95% CI, 23.63-162.66; χ^2 =132.775 ; P < 0.001) polymorphism was significantly associated with breast cancer. The association was also significant between breast cancer risk and *TOX3* (rs8051542) TC and *FGFR2* (rs1219648) AG variants.

Conclusion: Our findings suggested that genetic variants of *FGFR2* (rs1219648 AG) and *TOX3* (rs8051542 TC) can be potential candidate biomarkers for breast cancer risk.

Introduction

Breast cancer (BC) is the most frequently diagnosed malignancy among women worldwide.[1] Also, BC is one of the most common types of malignancy among Iranian women.[2] Recent epidemiologic studies have shown that the incidence of BC is rising in Iran.[3] As with other types of cancer, BC arises from a complex interaction between genetic and environmental risk factors. Predisposing genes such as BRCA1/2 and *FGFR2* are the main genetic factors, while environmental factors include lifestyle, tobacco smoking, occupational exposures, and hormonal changes in the body.[4,5]

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Several studies have suggested that different single nucleotide polymorphisms (SNPs) are associated with BC risk.[6,7] Recent studies revealed associations between single-nucleotide polymorphisms (SNPs) in *TOX3* and *FGFR2* and breast cancer risk, and genome-wide association studies in European, Asian, and African-American populations validated these findings.[8,9] Located on chromosome 16q12, *TOX3* contains a putative high-mobility group box motif, suggesting that it may act as a transcription factor in carcinogenesis and seems a novel breast cancer susceptibility loci.[10] *FGFR2* is a member of the fibroblast growth factor receptor family with a highly conserved sequence.[11] Although many studies have reported the association between *TOX3* and *FGFR2* SNPs and BC risk, because of the differences in regional and other environmental factors, the conclusions of related reports are still uncertain.[12,13]
The aim of this study was to evaluate two SNPs

(rs8051542 in TOX3 gene and rs1219648 in *FGFR2* gene) and their association with BC risk. Also, the assessment of three markers including human epidermal growth factor receptor 2 (HER2), progesterone receptor (PR), and estrogen receptor (ER) was conducted in this study.

Methods

Study Design and Patients

In this study, 126 women with grade 4 breast tumors and 160 healthy controls were examined. All patients were provided with informed consent. Peripheral blood samples (3 mL) were collected from patients using K3EDTA as an anticoagulant.

DNA Extraction

DNA isolation from blood samples was performed using FelxiGene DNA extraction kit (Qiagen, Germany). The concentration of the isolated DNA was quantified using a NanoDrop spectrophotometer (Thermofisher, USA).

Genotyping

Tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method was used to identify the genotypes of DNA samples. The DNA sequences of the SNPs rs8051542 and rs1219648 were obtained from the NCBI SNP database and used for designing the primers. Sequences of the primers have been shown in Table 1.

Immunohistochemistry Study

Breast carcinoma tissues were obtained from the Pathology Department of Rare Diseases Medical Center and Hazrat-e-Rasoul Medical Complex, Tehran, Iran. Immunohistochemistry study was performed to investigate ER, PR, and HER2 status.

Statistical Analysis

We assessed the Hardy-Weinberg equilibrium using the chi-squared (χ^2) test. The data were tested for association between the gene variations and breast cancer risk. The difference in the allele and genotype frequency between cancer patients and healthy controls was determined using standard χ^2. The odds ratios (OR) along with their 95% confidence intervals (95% CI) were also calculated.

Table 1. Inner and Outer Primer Sequences Used in the Tetra-primer ARMS-PCR Method

TOX3 (rs8051542)			bp
F-inner	CATGTGTTTTAAACATTTAGGTTATTAGAGTAC	C	215
R-inner	TGCTCCAATCATAGTGCTTCA	T	168
F-outer	CCAAACAGAAGAGATTCTGCTATATTTA		329
R-outer	GTGATATTATTGCTTCATATGATCGAAT		
FGFR2 (rs1219648)			
F-inner	TCTAAAGCACGCCTATTTTACTTGACACCCG	A	224
R-inner	AGCCATGGCCATCCTTGAAGCGT	G	164
F-outer	TCCACAATGGCGCAGAATTACTTACAGTATTCC		334
R-outer	GGTGATCCTTCACGTCTTGAAGATGTCTCC		

Table 2. Genotype Frequencies for Cases and Controls

SNP	Genotype	Cases, (N= 126)	%	Controls (N= 160)	%
TOX3 (rs8051542)					
	CC	57	45.238	36	22.5
	TC	50	39.682	106	66.25
	TT	19	15.07	18	11.25
FGFR2 (rs1219648)					
	AA	14	11.11	9	5.625
	AG	10	85.71	146	91.25
	GG	84	3.174	5	3.125

Results

The present study evaluated the association between *TOX3* (rs8051542) and *FGFR2* (rs1219648) with breast cancer risk. We also examined the relationship between these genotypes and three hormonal markers and their association with breast cancer risk factors (Table 3, Table 4, and Figure 1).

In the current study, *TOX3* (rs8051542) CC showed a significant association (OR =1.24, 95% CI , 0.72-2.14; χ^2 =16.608 ; P < 0.001) and *FGFR2* (rs1219648) GG; (OR = 62.0; 95% CI, 23.63-162.66; χ^2 =132.775 ; P < 0.001) with breast cancer.

However, there was a strong, inverse association between the low-risk allele and tumor markers (Table 3) and similar to *TOX3* (rs8051542) TC (OR = 0.34; 95% CI, 0.21-0.54; χ^2 =9.897; P = 0.002) and *FGFR2* (rs1219648)AG; (OR = 0.01, 95% CI, 0-0.02; χ^2 = 197.342; P < 0.001)(Table 3,

n Between Genotypes

SNP	Genotype	OR	95% CI	χ^2	P value
TOX3 (rs8051542)					
	CC	1.24	0.72-2.14	16.608	**<0.001**
	TC	0.34	0.21-0.54	9.897	**0.002**
	TT	1.4	0.7-2.8	0.918	0.338
FGFR2 (rs1219648)					
	AA	2.1	0.88-5.02	2.869	0.090
	AG	0.01	0-0.2	197.342	**<0.001**
	GG	62.0	23.63-162.66	132.775	**<0.001**

Table 4. Immunohistochemical Analysis of HER2, ER, and PR Status

	ER+	ER−	PR+	PR−	HER2+	HER2−
TOX3 (rs8051542)						
CC	15	5	15	5	7	10
TC	22	2	22	2	11	13
TT	11	2	11	2	2	11
P value		0.64		0.11		0.81
FGFR2 (rs1219648)						
AA	7	0	7	0	3	4
AG	36	9	36	9	15	29
GG	2	0	2	0	2	0
P value		**0.003**		**0.003**		0.2

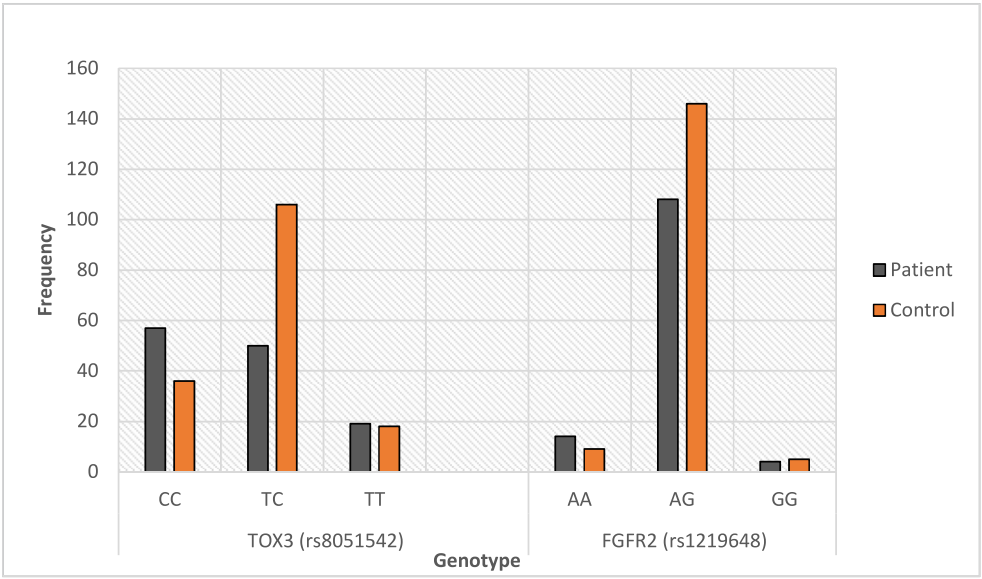

Figure 1. Frequencies of Genotypes in Cases (n = 126) and Controls (n = 160)

and Figure 1). This may be attributable to significant polymorphisms of SNPs variants at low risk (Table4).

FGFR2 (rs1219648) AG was associated with ER-positive and PR-positive tumor phenotypes. Also, the association of *TOX3* (rs8051542) TC variant with PR- and ER-positive tumors was stronger than that of CC and TT variants.

Discussion

Recently, studies have indicated several SNPs as novel independent loci for diagnosis and prognosis of breast cancer.[14] The aim of the present study was to explore the association of SNPs in *FGFR2* and TOX3 genes with breast carcinoma subtypes. Previous reports focused on intronic SNPs in FGFR2 and proposed the gene to be a candidate locus for breast cancer.[15] Also, it was reported in similar studies that rs8051542 was significantly correlated with breast cancer risk.[16] Samson *et al* examined the *FGFR2* SNPs in southern India and showed that there was no association between *FGFR2* (C906T polymorphism) and breast cancer in this Asian population.[17] Another study genotyped rs2981582, rs1219648, rs2981578, and rs7895676 polymorphisms in *FGFR2* in breast cancer patients from northern India. The results showed that SNPs in intron 2 of *FGFR2* may contribute to genetic susceptibility to breast cancer in northern Indian population.[18] Zhang *et al* studied three SNPs in

FGFR2 gene, including rs1219648, in a meta-analysis in China. Results of this study indicated that these polymorphisms are significantly associated with the BC risk.[19]

We observed that *FGFR2* (rs1219648) AG polymorphism is associated with ER- and PR-positive tumors. Also, the association of *TOX3* (rs8051542) TC variant with PR- and ER-positive tumors was stronger compared with CC and TT variants. These findings are consistent with several studies of *FGFR2* SNPs in BC and ER- and PR-positive tumor subtypes.

Therefore, both the high-risk and low-risk alleles (CC and TC, respectively) of *TOX3* SNP (rs8051542) are associated with BC risk. In addition, the association of TC, CC, and TT alleles in *TOX3* SNP (rs8051542) with ER+ and PR+ tumors was observed.

As conclusion, these findings suggest that genetic variants of *FGFR2* (rs1219648 AG) and *TOX3* (rs8051542 TC) can be potential candidate biomarkers for breast cancer risk.

Conflict of Interest

The authors have nothing to disclose.

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