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Estrogen Receptor-α Gene, Codon 594 (G3242A) Polymorphism Among Iranian Women with Breast Cancer: A Case Control Study

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Abstract: A case-control study was conducted to establish a database of ESR1 polymorphisms in Iranian population in order to compare Western and Iranian (Middle East) distributions and to evaluate ESR1 polymorphism as an indicator of clinical outcome. The *ESR1* gene was scanned in Iranian patients newly diagnosed invasive breast tumors, (150 patients) and in healthy individuals (147 healthy control individuals). PCR single-strand conformation polymorphism technology and direct sequencing was performed. The silent single nucleotide polymorphism (SNPs) was fonnd, as reported previously in other studies, but at significantly different frequencies. The frequency of genotype 01 in codon 594 (ACG→ACA), (G3242A), exon 8 was significantly higher in breast cancer patients (48.0%) than in control individuals (1.4%; p = 0.001). The allele 1 in codon 594 was significantly more common in breast cancer patients with age at menarche </=12 (40.8%) than in those which their menstruation began at older than 12 years old (23.9%; p = 0.002). The allele 1 in codon 594 exhibited, the greater the frequency, the lesser the likelihood of LN metastasis. Present results demonstrated that this particular SNP marker may increase accuracy in predicting LN. Therefore, this SNP marker further increased predictive accuracy in Iranian population. These data suggest that ESR1 polymorphisms are correlated with various aspects of breast cancer in Iranian *ESR1* genotype, as determined during pre-surgical evaluation, might represent a surrogate marker to increase predicting breast cancer in Iranian population.

Key words: Breast cancer, estrogen receptor-α, polymorphism, lymph node metastases PCR-SSCP

INTRODUCTION

Breast cancer is amongst leading causes of death in women worldwide, every three minutes a woman in the United States is diagnosed with breast cancer. It is the most common cancer among Iranian women with more than 7000 new diagnosed in each year. Unfortunately, the current criteria can ouly help 60% of women with breast cancer in diagnosis and long-term treatment. Breast cancer affects Iranian women at least one decade yonnger than their counterparts in developed countries (Harirchi *et al*., 2004; Lin *et al*., 2008). The mortality rate of breast cancer was 5.8 per 100,000 women in Tehran (Mousavi *et al*., 2007).

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Estrogens play a crucial role in the pathogenesis and progression of breast cancer. The effects of estrogens are mediated primarilythrough intracellular estrogen receptors (ER). To date, there are two known types of ERs, *ESR1* and *ESR2*. The *ESR1* gene is localized on chromosome 6q25.1 (Menasce *et al*., 1993) and the *ESR2* gene is localized on chromosome 14q22-24 (Enmark *et al*., 1997). The importance of estrogen in breast cancer development is also supported by studies demonstrating the occurrence of marked changes in estrogen signaling and in the expression of the two estrogen receptors (ERs), ER alpha and ER beta, during breast tumorigenesis and progression (Murphy *et al*., 1997; Dotzlaw *et al*.,1997; Hu *et al*., 1998; Iwao *et al*., 2000; Henderson and Feigelson, 2000; Herynk and Fuqua, 2004).

Available evidence suggests that breast cancer might result from interactions between genetic elements and a variety of possible environmental factors. Ethnicity also plays a role in risk for breast cancer, with the incidence varying from lowest in certain groups of Asian women to highest in Caucasian women (Brinton *et al*., 2002). Asian-Americans have traditionally had the lowest risk for breast cancer in the USA, although the difference diminishes over a couple of generations (Brinton *et al*., 2002). Comparison of incidence-age curves for breast cancer in Asian and Western genomic populations in their native countries reveals an additional interesting difference. Age distributions for East and Middle Asian groups exhibit an inverted 'V' shaped curve, with the peak in the age range 40-50 years, contrasting with the continued increasing incidence beyond the age of 50 years in Western women. The similar and apparently unique manifestation of breast cancer in genetically similar but geographically separated race groups suggests the involvement of an unusnal genetic factor in different populations (Hsiao *et al*., 2004).

Mutation and polymorphism of cancer-associated genes have been found to predict tumor formation and prognosis. It is also considered as an effective risk factor with positive effects (Vasconcelos *et al*., 2002; Heldring *et al*., 2007; Wang *et al*., 2007; Holst *et al*., 2007) and negative effects (Slattery *et al*., 2007; Gonzalez-Zuloeta Ladd *et al*., 2008; Einarsdóttir Darabi *et al*., 2008) in the different studies.

The hnman *ESR1* gene exhibits low mutational frequency in breast cancer tissue. However, *ESR1* allelic variant have been associated with breast cancer risk in Caucasians, as have certain clinical features including presence of a family history and Lymph Node (LN) metastasis (Roodi *et al*., 1995; Iwase *et al*., 1996; Curran *et al*., 2001; Vasconcelos *et al*., 2002).

At present the literature contains little information regarding *ESR1*gene expression, mutational frequency and allelic variants in breast cancer among Asians, especially those who reside in their native country. Thus, the present study examined polymorphism in *ESR1* exon 8 among an Iranian clinical group of breast cancer patients in order to establish a genetic polymorphism database for the ESR1encoding region of the Iranian genome, to compare this distribution with that reported for Western and other Asian study groups and to test for any correlation between exon 8 of ESR1 polymorphism and various clinically observable features of breast cancer in Iranian women.

MATERIALS AND METHODS

Study Population

A case-control study was conducted from April 2004-September 2007 in Tehran, Iran. The breast cancer patients (n = 150; median age 47.49±11.43 years) were newly diagnosed and mostly living in Tehran. Patients enrolled into the study if they had a confirmed pathological breast cancer diagnosis at the Imam Khomeini Hospital Complex (a large teaching and general hospital in the central district of Tehran) and were referred to the several clinics of the Cancer Institute, including Women Sections 1 and 3 and Central Clinics of 1 and 2 for breast surgery. The control group (n = 147; median age 40.75±10.54 years) included healthy women neither with any history of breast cancer nor any other neoplastic diseases and also none of their relatives had a history of breast cancer. Women with hysterectomy and artificial menopause or exposed to any kind of radiation and chemotherapy in their

life time were excluded from the study. By the permission from the hospital ethics committee, all the patients provided with written informed consent to participate in that protocol before entering into the present study.

Demographical and risk factor data were collected using a short structured questionnaire, including information on age, weight, height, race, religion, marital status, number of pregnancies and children, age at the first child birth, average lactation term, family history of breast cancer (first-degree relatives), age at menarche, age at marriage, parity, age at first pregnancy, menopausal status and age at menopause, ABO and Rhesus blood groups, race, age at onset, lymph node metastases, cancer stage at the time of testing and ER expression in breast cancer tissue. An ongoing protocol to collect and store blood samples for future genomic tests had been approved by the institutional review board. Peripheral whole blood was collected and kept in storage at -80°C nntil genotyping analysis. This information was obtained by interview with patients and family members.

Screening for ESR1 Variants by Single Strand Conformation Polymorphism Analysis

In order to identify any mutation or variant sites in the Iranian population, the strategy was to screen initial samples for the entire coding region of *ESR1* using the PCR single-strand conformation polymorphism (SSCP) method. A total of 150 breast cancer patients were screened at this stage and compared with 147 control individuals in order to identify disease-associated variants/ mutations. Genomic DNA was extracted from whole blood cells using DNG™-Plus extraction solution kit (Cinnagen Inc., Tehran, Iran) in accordance with the mannfacturer's instructions. Genomic DNA (50 ng) was used for each run of PCR-based genotyping.

Exon 8 of the *ESR1*gene was amplified by PCR methods, using set of primers according to the oligonucleotide sequences by Hsiao *et al*. (2004):

Forward primer 5` -CTGTGTCTTCCCACCTACAG -3` (337-356)
Reverse primer 5` -GGGTAAAATGCAGCAGGGATT- 3` (641-621)

PCR was performed for 30 cycles of 30 sec at 95°C, 30 sec at 58°C and 40 sec at 72°C. Optimal electrophoretic separation for SSCP was conducted in 8% polyacrylamide gel (19:1 Acrylamide: Bisacrylamide) in buffer (90 mmol L^{-1} Tris-borate and 2 mmol L^{-1} EDTA) at 200 V for 2 h followed with 250 V for 24 h at 16°C. After electrophoresis, the bands on gel were visualized using 0.1% silver nitrate stain. PCR samples exhibiting varying band shifting patterns as the result of first sequencing with forward primer, re-purified on agarose gel using a DNA Extraction Kit, Fermentas No. K0153, Germany and directly sequenced by big dye Terminator V3.1 Cycle Sequencing kit protocol, (Applied Biosystem Kit, Microgen Co., USA), on a sequencer ABI 3130XL (16 capillaries).

Also, the PCR products purification method was performed in order to confirm sequencing by reverse primer. The PCR products were purified using QIA quick PCR purification Kit (50), QIAGEN cat. No. 28104, USA (through Zistbaran Co. Iran).

Statistical Analysis

χ^2 testing was employed to assess the influence of polymorphism status on features of breast cancer. Unconditional logistic regression analysis was performed using SPSS software (version 11.5 for Windows XP; SPSS Inc., Cary, NC, USA) to calculate odds ratios (ORs) with 95% confidence intervals (CIs) and to examine the predictive effect of each factor on risk for breast cancer. $p<0.05$ was considered as a statistically significant.

RESULTS

Table 1 presents frequencies distribution of selected demographic characteristics and major risk factors such as BMI, age beginning at menstruation race, ABO and Rhesus blood groups in the study

Table 1: Frequencies distribution of selected demographic characteristics and major risk factors in the study population: breast cancer versus control groups

	Case		Control		Total		
Characteristics	Frequency	%	Frequency	%	Frequency	%	Test result
Age (years)							
</ = 40	52	41.3	98	57.3	150	50.5	$\chi^2 = 7.417$
>40	74	58.7	73	42.7	147	49.5	p = 0.006
Total	126	100.0	171	100.0	297	100.0	
BMI (kg m^{-2})							
</ = 18.5 (underweight)	5	3.3	9	6.1	14	4.7	$\chi^2 = 21.663$
18.6-24.9 (normal)	57	38.0	90	61.2	147	49.5	p = 0.001
25-29.9 (overweight)	55	36.7	35	23.8	90	30.0	
>30 (obese)	33	22.0	13	8.9	46	15.5	
Total	150	100.0	147	100.0	297	100.0	
Profession							
Housewife	129	86.0	27	18.3	156	52.5	$\chi^2 = 137.642$
Student	2	1.3	32	21.8	34	11.5	p = 0.001
Others	19	12.7	88	59.9	107	36.0	
Total	150	100.0	147	100.0	297	100.0	
Religion							
Moslem	148	98.7	146	99.3	294	99.0	$\chi^2 = 0.136$
Non- Moslem	2	1.3	1	0.7	3	1.0	p = 0.574
Total	150	100.0	147	100.0	297	100.0	
Age at menarche (years)							
</ = 12	60	40.0	36	24.5	96	32.3	$\chi^2 = 8.165$
>12	90	60.0	111	75.5	201	67.7	p = 0.004
Total	150	100.0	147	100.0	297	100.0	
Marital status							
Married	140	93.3	99	67.3	239	80.5	$\chi^2 = 11.992$
Single	10	6.7	48	32.7	58	19.5	p = 0.001
Total	150	100.0	147	100.0	297	100.0	
Age at marriage (years)							
</ = 20	92	65.7	40	40.4	132	55.2	$\chi^2 = 14.962$
>20	48	34.3	59	59.6	107	44.8	p = 0.001
Total	140	100.0	99	100.0	239	100.0	
No. of deliveries (married individuals)							
0	6	4.3	5	5.1	11	4.6	$\chi^2 = 41.493$
1	9	6.4	37	37.4	46	19.3	p = 0.001
2	21	15.0	29	29.2	50	20.9	
>/ = 3	104	74.3	28	28.3	132	55.2	
Total	140	100.0	99	100.0	239	100.0	
No. of children (married individuals)							
0	6	4.3	5	5.1	11	4.6	$\chi^2 = 38.285$
1	10	7.1	38	38.4	48	20.1	p = 0.001
2	30	21.4	31	31.3	61	25.5	
>/ = 3	94	67.2	25	25.2	119	49.8	
Total	140	100.0	99	100.0	239	100.0	
Menopause status							
Yes	59	39.3	18	12.2	77	25.9	$\chi^2 = 28.367$
No	91	60.7	129	87.8	220	74.1	p = 0.001
Total	150	100.0	147	100.0	297	100.0	
Race							
Arab and Armani	3	2.0	-	-	3	1.0	$\chi^2 = 7.351$
Fars	60	40.0	88	59.9	148	49.8	p = 0.007
Lor and Kurdish	18	12.0	9	6.1	27	9.1	
Turkish	46	30.7	39	26.5	85	28.6	
Gilaki and Mazani	23	15.3	11	7.5	34	11.5	
Total	150	100.0	147	100.0	297	100.0	
ABO and Rh blood groups							
A^+	27	18.0	39	26.5	66	22.2	$\chi^2 = 25.144$
B^+	12	8.0	31	21.1	43	14.5	p = 0.023
AB^+	6	4.0	15	10.2	21	7.1	
O^+	100	66.7	47	32.0	147	49.5	
A^-	-	-	4	2.7	4	1.4	
B^-	2	1.3	4	2.7	6	2.0	

Table 1: Continued

Characteristics	Case Frequency	Case %	Control Frequency	Control %	Total Frequency	Total %	Test result
AB^-	-	-	1	0.7	1	0.3	
O^-	3	2.0	6	4.1	9	3.0	
Total	150	100.0	147	100.0	297	100.0	
ABO blood groups							
A	27	18.0	43	29.2	70	23.6	$\chi^2 = 33.201$
B	14	9.3	35	23.8	49	16.5	$p = 0.001$
AB	6	4.0	16	10.9	22	7.4	
O	103	68.7	53	36.1	156	52.5	
Total	150	100.0	147	100.0	297	100.0	
Rh blood groups							
Positive	145	96.7	132	89.8	277	93.3	$\chi^2 = 5.813$
Negative	5	3.3	15	10.2	20	6.7	$p = 0.016$
Total	150	100.0	147	100.0	297	100.0	
Family history of breast cancer							
First-degree family affected	19	12.7	-	-	19	6.4	$\chi^2 = 19.893$
Not affected	131	87.3	147	100	278	93.6	$p = 0.001$
Total	150	100.0	147	100	297	100.0	
First-degree family history of breast cancer							
Mother	8	5.3	-	-	8	2.7	$\chi^2 = 27.232$
Sister	6	4.0	-	-	6	2.0	$p = 0.001$
Daughter	4	2.7	-	-	4	1.4	
Mother and sister	1	0.7	-	-	1	0.3	
Not affected	131	87.3	147	100	278	93.6	
Total	150	100.0	147	100	297	100.0	

population comprising between breast cancer and control groups. All these characteristics with different frequencies distribution between breast cancer and control groups were statistically significant ($p<0.05$).

Allelic frequencies of exon 8 in the *ESR1* gene among 297 Iranian women (150 breast cancer patients and 147 healthy control individuals) was screened for mutation or variant sites of single nucleotide polymorphisms (SNPs) by PCR-SSCP and DNA sequencing. The observed numbers of individuals with different genotypes showed that SNP fitted the Hardy–Weinberg equilibrium for both control and patient groups ($p>0.05$).

The encoding region exon 8 of the *ESR1* gene of 150 breast cancer patients and 147 healthy individuals was screened for mutation or variant sites by PCR-SSCP and DNA sequencing. This stage of testing revealed no novel mutations but it did reveal the presence, in the Iranian population studied, one silent single nucleotide polymorphisms (SNP), codon 594 (ACG-ACA), rs2228480 (dbSNP128), nucleotide G is converted to A at 594 with the significant frequencies of 48.0% in cancer patients and 1.4% in control individuals. Both ACG and ACA are codon which code for Threonine amino acid, that have previously been reported in earlier study groups.

The frequency of genotype 00, normal (ACG/ACG) was found 45.3% in case and 98.6% (twofold higher) in control groups, but heterozygote genotype, 01 (ACG/ACA) the frequency was significantly less in control individuals (1.4%) than in cancer patients (48.0%) ($\chi^2 = 27.035$; $p = 0.001$). The genotypic frequencies for genotype 11, homozygote (ACG/ACG) was found only in cancer patients (6.7%) (Table 2).

The frequency of allele 1 (ACG) in codon 594 was significantly much higher (more than forty three fold) in cancer patients (30.7%) than in control individuals (0.7%) ($\chi^2 = 100.232$; $p = 0.001$).

The genotypic frequency of genotype 01, heterozygote, in codon 594 was significantly higher (48.3%) in cancer patients with age at menarche 12 years old or below, as one of important risk factor in developing breast cancer, than those with age at menarche above 12 years old. Besides, all homozygote individual for codon 594 (ACA/ACA) were within breast cancer individual with age at menarche 12 years old or below ($\chi^2 = 17.358$; $p = 0.001$).The allelic frequency of allele 1 (ACA) in codon 594 was significantly higher (two fold) in cancer patients with age at menarche 12 years old or

Table 2: Genotypic and allelic frequencies of estrogen receptor-α exon 8, codon 594 (ACG /ACA); in the study population: breast cancer versus control groups and breast cancer cases in the presence versus the absence of major risk factors

		ER-α genotypes			ER-α alleles	
Characteristics		00[a]	01[b]	11[c]	0[d]	1[e]
Breast cancer						
Case	(n = 150)	68(45.3%)	72(48.0%)	10(6.7%)	208(69.3%)	92(30.7%)
Control	(n = 147)	145(98.6%)	2(1.4%)	-	292(99.3%)	2(0.7%)
		$\chi^2 = 27.035$, p = 0.001			$\chi^2 = 100.232$, p = 0.001	
Age at menarche (years)						
</ = 12	(n = 60)	21(35.0%)	29(48.3%)	10(16.7%)	71(59.2%)	49(40.8%)
>12	(n = 90)	47(52.2%)	43(47.8%)	-	137(76.1%)	43(23.9%)
		$\chi^2 = 17.358$, p = 0.001			$\chi^2 = 9.723$, p = 0.002	
Race						
Arab and Armani	(n = 3)	-	1(33.3%)	2(66.7%)	1(16.7%)	5(83.3%)
Fars	(n = 60)	23(38.3%)	31(51.7%)	6(10.0%)	77(64.2%)	43(35.8%)
Lor and Kurdish	(n = 18)	10(55.6%)	7(38.9%)	1(5.5%)	27(75.0%)	9(25.0%)
Turkish	(n = 46)	24(52.2%)	21(45.7%)	1(2.1%)	69(75.0%)	23(25.0%)
Gilaki and Mazani	(n = 23)	11(47.8%)	12(52.2%)	-	34(73.9%)	12(26.1%)
		$\chi^2 = 9.602$, p = 0.002			$\chi^2 = 5.494$, p = 0.019	
Family history of breast cancer						
First-degree family affected	(n = 19)	7(36.8%)	11(57.9%)	1(5.3%)	25(65.8%)	13(34.2%)
Not affected	(n = 131)	61(46.6%)	61(46.6%)	9(6.8%)	183(69.8%)	79(30.2%)
		$\chi^2 = 0.854$, p = 0.652			$\chi^2 = 0.257$, p = 0.612	
Lymph node metastases						
Yes	(n = 23)	11(47.8%)	10(43.5%)	2(8.7%)	32(69.6%)	14(30.4%)
No	(n = 127)	57(44.9%)	62(48.8%)	8(6.3%)	176(69.3%)	78(30.7%)
		$\chi^2 = 0.321$, p = 0.852			$\chi^2 = 0.001$, p = 0.97	

[a]: Genotype 00 (normal), ACG/ACG, [b]: Genotype 01 (heterozygote), ACG/ACA, [c]: Genotype 11 (homozygote), ACA/ACA; [d]: Allele 0, ACG, [e]: Allele 1, ACA

below than those with age at menarche above 12 years old ($\chi^2 = 9.723$, p = 0.002). Other significant differences in genotypic and allelic frequencies were found for races. The genotypic frequency of genotype 01, heterozygote, in codon 594 was significantly higher in Fars (51.7%) and Gilaki and Mazani 52.2%) cancer patients races than the others, instead, the genotypic frequency of genotype 11, homozygote, in codon 594 was significantly higher in Arab and Armani (66.7%) cancer patients than the other races ($\chi^2 = 9.602$, p = 0.002,). The allelic frequency of allele 1 (ACA) in codon 594 was significantly higher (about three fold) in Arab and Armani cancer patients (83.3%) than the other races ($\chi^2 = 5.494$, p = 0.019).

ER-α genotypes in codon 594 were compared with selected clinical breast cancer features, including age at menarche, onset age, parent's marriage status, ABO and Rh blood groups, first-degree of family history of breast cancer, LN metastasis, other cancer affected status and type of breast cancer. The only significant correlation was found for age at menarche as indicated by the ORs presented in Table 2.

In consideration of breast cancer development and genotype frequencies for codon 594, it was exhibited a different distributions in the case and control groups, with the statistical significance of p = 0.001. The estimated risk was much higher (thirty-six fold) in breast cancer patients than control individuals who were 01 heterozygote codon 594 (OR 0.013, 95% CI 0.003- 0.055), in compare with corresponding 00 homozygote were within the cancer patients (100%).

The only significant correlation was found for age at menarche (p = 0.001). Genotype frequencies exhibited different distributions in the age at menarche 12 years old and below and above 12 years old; the estimated risk was almost the same for individuals who were 01 homozygote in codon 594 (OR 0.663, 95% CI 0.330-1.331) than for the corresponding 00 homozygote with two fold lower for individuals with age at menarche 12 years old and below than above 12 years old. Besides, all individuals who were 11 homozygote in codon 594 had age at menarche 12 years old and below.

DISCUSSION

Receptor-mediated estrogen activation participates in the development and progression of breast cancer. Evidence suggests that alterations in estrogen signaling pathways, including estrogen receptor-α (*ESR1*) occur during breast cancer development. *ESR1* gene polymorphism has been found to be associated with breast cancer and clinical features of the disease in Caucasians. Epidemiologic studies have revealed that age–incidence patterns of breast cancer in Middle East differ from those in Caucasians. Genomic data for *ESR1* in either population is therefore of value in the clinical setting for that ethnic group. Whether polymorphisms in the ESR1 are associated with breast cancer risk was also investigated.

The association of *ESR1* genetic polymorphisms with breast cancer risk attracts much attention because ER functions as a hormone-dependent transcriptional regulator, which, in turn, plays a significant role in the development of breast cancer; therefore, breast cancer associated ESR1 polymorphisms were surveyed in earlier studies (Roodi *et al*., 1995; Iwase *et al*., 1996; Southey *et al*., 1998; Curran *et al*., 2001; Kang *et al*., 2002; Vasconcelos *et al*., 2002).

This study was conducted to establish a database of ESR1 polymorphisms in Iranian population in order to compare Western and Iranian (Middle East) distributions and to evaluate ESR1 polymorphism as an indicator of clinical outcome. Unexplained differences between Asian and Western breast cancer symptomatology and demographics led us to consider whether unknown genetic factors within the Iranian genome are involved, prompting us to conduct the present PCR analysis of ESR1 polymorphism. Although, the PCR-SSCP based genotyping was able to detect new mutations, none was found.

ESR1 gene (exon 8) screening was conducted in 150 consecutive breast cancer patients and 147 healthy women. PCR primers used in the initial screening in a US study conducted in Caucasians (13). However, the PCR-SSCP screening revealed the presence of the SNP-in 594 (ACG→ACA) (G/A,T3242T) a significant frequencies of allele 1 very much higher in the cases than controls (30.7 and 0.7%, respectively). In the Iranian population the allele 1 frequency was found more common than that were previously reported in USA (19.0%), Australia (24.0%) and Taiwan (18.5%) populations (Curran *et al*., 2001). Thus, the Iranian population exhibited a distinct pattern of *ESR1* gene polymorphism.

The allelic frequency of allele 1 (ACA) in codon 594 was significantly higher (two and threefold) in cancer patients with two important risk factors; age at menarche 12 years old or below and race (Arab and Armani) ($\chi^2 = 9.723$, $p = 0.002$ and $\chi^2 = 5.494$, $p = 0.019$, respectively).

LN metastasis is considered an important indicator when deciding whether chemotherapy should be given (Carter *et al*., 1989; Henson *et al*., 1991; Fisher *et al*., 1993; Goldhirsch *et al*., 1995; Canavese *et al*., 1998). In this study the estimated risk for LN metastases was six fold lower for individnals who were 01 heterozygote in codon 594 (OR 1.196, 95% CI 0.473-3.029) and four fold lower for those who were 11 homozygote in codon 594 (OR 0.772, 95% CI 0.144-4.136) than for the corresponding 00 homozygote, although the differences were not significant ($p = 0.852$). Taking this, it was noted that the greater the frequency of allele 1, the lesser the likelihood of LN metastasis. These results demonstrated that this particular SNP marker may increase accuracy in predicting LN metastasis (Table 3). To the knowledge, the link between silent polymorphisms and phenotypes is unclear. One of the possibilities might be that the silent polymorphism is in linkage with another genetic mutation that directly affects breast cancer phenotype. The other possibility might be that the nucleotide composition at the silent polymorphic site could alter the gene expression level of ER-α, thus leading to the association to LN metastasis in breast cancer.

The frequency of allele 1 in codon 594 was much greater in the Iranian population studied here than in Western populations; this finding, together with the relatively low incidence of breast cancer in Iran, suggests that this SNP is also, protective against breast cancer.

Table 3: Estimated risk for selected demographic characteristics and major risk factors with estrogen receptor-α exon 8, codon 594 in different genotypes

Genotype	Yes (n = 150)	No (n = 147)	p-value	OR (95% CI)
Normal[a]	68 (31.9%)	145 (68.1%)	0.001	1.0 (reference)
Heterozygote[b]	72 (97.3%)	2 (2.7%)		0.013 (0.003-0.055)
Homozygote[c]	10 (100%)	-		-
Lymph nod metastases				
	(n = 23)	(n = 127)		
Normal	11 (16.2%)	57 (83.8%)	0.852	1.0 (reference)
Heterozygote	10 (13.9%)	62 (86.1%)		1.196 (0.473-3.029)
Homozygote	2 (20.0%)	8 (80.0%)		0.772 (0.144-4.136)

[a]: Genotype normal or 00, ACG/ACG, [b]: Genotype heterozygote or 01, ACG/ACA, [c]: Genotype homozygote or 11, ACA/ACA

CONCLUSION

In conclusion, *ESR1*, exon 8 polymorphisms in Iranian breast cancer women (150 breast cancer patients and 147 control individuals) were established using PCR- SSCP of peripheral blood. The same SNP reported in earlier studies were found in the Iranian population studied, but at different frequencies than in Western studies. Small but statistically significant correlations were found between allele distribution and individual and familial manifestation of breast cancer. Because of the limited sample size in the present study, the observed correlation between LN metastasis and allele 1 of codon 594 will require further confirmation. This is planned as part of the future research, because SNP determination from peripheral blood represents a highly feasible and noninvasive option for preoperative evaluation.

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