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Original Article
Estrogen receptor genes variations and breast cancer risk in Iran

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Abstract: Evidence suggests that alterations in estrogen signaling pathways, including estrogen receptor α (ER-α) and estrogen receptor β (ER-β) occur during breast cancer development. ER-α and ER-β genes polymorphisms have been found to be associated with breast cancer and clinical features of the disease in the western countries. In the current study, we evaluated the hypothesis that certain sequence variants of the ER-α and ER-β genes are associated with an additively increased risk for breast cancer in Iranian women breast cancer patients. The genes were scanned in 150 Iranian patients with newly diagnosed invasive breast tumors and in healthy control individuals by PCR single-strand conformation polymorphism (SSCP) method. Three single nucleotide polymorphisms (SNPs) in codon10 (TCT→TCC), codon 352 (CCG→CCC) and codon 594 (ACG→ACA) in ER-α gene and one SNP codon 392 (CTC→CTG) in ER-β were revealed have additive effects in developing breast cancer and LN metastases. Also, SNP in codon 392 of estrogen receptor-β gene is more effective (threefold) than those SNPs in codons 10, 325, 594 of estrogen receptor-α gene in developing LN metastases in breast cancer patients. SNPs in estrogen receptor α and β have additive effects in increasing risk for developing breast cancer with LN metastases among Iranian women breast cancer patients.

Keywords: Breast cancer, estrogen receptor, LN metastases, polymorphism

Introduction

Breast cancer is the most common cancer among women worldwide [1]. Incidence rates increased rapidly predominantly in women 50 and older [2] in the 1980s due to the increased detection of smaller, earlier-stage cancers with the widespread adoption of the screening of mammography among asymptomatic women [3], with the prevalence rate of 120 per 100,000 [4]. A portion of this increase can be attributed to changes in reproductive patterns, such as delayed childbearing and having fewer children (increased life expectancy). Though at one of the lowest incidence rates in Iran rather than other Asian countries, during last four decades, increasing its incidence rate has made breast cancer one of the most frequent malignancies among Iranian women [5]. Breast cancer affects Iranian women at least one decade younger than their counterparts in developed countries [6]. The mortality rate of breast cancer was 5.8 per 100,000 women in Tehran in 1998 [4], 2.5 per 100,000 for the female population, and 7762 years life lost in the 18 provinces of Iran in 2001 [7]. Developing countries hope to be on the threshold of eliminating breast cancer as a major public health threat [8]. Early detection of breast cancer remains an important challenge to health professionals. According to the World Health Organization's recommendations on implementing national cancer control programs [9], assessment of the magnitude of the cancer problem (i.e., incidence, prevalence, and mortality) is the first step in this process.

It is known that breast cancer typically arises in luminal epithelial cells of the mammary gland [10, 11]. These cells contain estrogen receptors (ERs), which respond to ovarian estrogen in normal mammary gland development. How estrogens stimulate cell growth is not fully understood, but it is known that estrogen activation of ER results in transcription of various genes that

are involved in cellular proliferation.

Only a small fraction (<5%) of women diagnosed with breast cancer have a clear hereditary predisposition [12-14], and about one half have predisposing mutations in *BRCA1*, *BRCA2*, *PTEN*, *TP53*, or other known cancer predisposing genes. However, twin studies indicate that the heritability of breast cancer is about 30% [15], suggesting that genes other than the well-mapped regions act as modifiers of breast cancer risk. Although it is likely low penetrance as well as high penetrance genes may be involved in the etiology, it remains unclear which genomic regions and which biochemical functions or signal transduction pathways account for the additional, heritability of breast cancer incidence or progression.

The human ER-α gene exhibits low mutational frequency in breast cancer tissue [16]. However, ERs allelic variants have been associated with breast cancer risk [17-28] in Caucasians, as have certain clinical features including presence of a family history [16] and lymph node (LN) metastasis [24]. Evidence is accumulating to suggest that breast cancer is a collection of biologically distinct disease subtypes characterized by unique gene expression profiles, molecular or protein markers, and that exhibit variable clinical behavior, prognosis, and response to therapies [29-34].

In the current study, we evaluated the hypothesis that certain sequence variants of the *ER-α* and *ER-β* genes are associated with an additively increased risk for breast cancer. This study includes the following components: (*a*) direct sequencing of the selected exons variations, among all of the cases and controls whose DNA samples were available at the time of this study; and (*d*) assessment of the association of ERs sequence variants and their interaction with each others on breast cancer risk.

Methods

Study population

A case-control study was conducted with the breast cancer patients (*n* =150; mean age 47.49 ± 11.43 years) were newly diagnosed and mostly living in Tehran. They were entered 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 our 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; mean age 40.75 ± 10.54 years) included healthy women neither with any history of breast cancer nor any other neoplastic diseases, also none of their relatives had a history of breast cancer at the same clinic. Women with hysterectomy and artificial menopause or exposed to any kind of radiation and chemotherapy in their life time were excluded from the study. All patients provided written informed consent to participate before entering into the present study.

Demographical and risk factor data were collected using a short structured questionnaire, including information on family history of breast cancer (first-degree relatives), age at menarche, lymph node metastases (LN) (**Table 1**). An ongoing protocol to collect and store blood samples for future genomic tests had been approved by the institutional review board. Peripheral blood was collected and genotyping analysis was performed for selected regions in both genes.

*Screening for ER-*α and *ER-β variants by single strand conformation polymorphism analysis*

To identify any variant site on these genes in the Iranian population, our strategy was to screen samples for the selected coding regions of *ER-α* and *ER-β* 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 manufacturer's instructions. Genomic DNA (50 ng) was used for each run of PCR-based genotyping.

Exons 1, 4 and 8 of the *ER-α* gene were amplified by PCR methods, using set of primers according to the oligonucleotide sequences in Hsiao *et al*. [35] (**Table 2**). PCR was performed for 30 cycles of 30 seconds at 95°C, 30 seconds at 58°C and 40 seconds at 72°C. Optimal electrophoretic separation for SSCP was conducted in Polyacrylamide gel (8% for exon 1 and

Table 1. The distributions of selected demographic characteristics and major risk factors for breast cancer of whole study population: breast cancer versus control groups.

Characteristics	Case Number (%)	Control Number (%)
Age (years):		
</=40	52 (41.3)	98(57.3)
>40	74(58.7)	73(42.7)
BMI(kg/m^2):		
</=18.5 (underweight)	5(3.3)	9(6.1)
18.6 - 24.9 (normal)	57(38.0)	90(61.2)
25.0 - 29.9 (overweight)	55(36.7)	35(23.8)
>30.0 (obese)	33(22.0)	13(8.9)
Age at menarche (years):		
</=12	60(40.0)	36(24.5)
>12	90(60.0)	111(75.5)
Age at menopause (years):		
</=50	47(79.7)	11(61.1)
> 50	12(20.3)	7(38.9)
Onset Age of breast cancer (years):		
<40	48(32.0)	-
>/=40	66(44.0)	-
After menopause	36(24.0)	-
Family history of breast cancer:		
First– degree family affected	19(12.7)	-
Not affected	131(87.3)	147(100)
First-degree family history of breast cancer:		
Mother	8(42.1)	-
Sister	6(31.6)	-
Daughter	4(21.0)	-
Mother & sister	1(5.3)	-
Lymph node metastases:		
Yes	23(15.3)	-
No	127(84.7)	-
First-degree family history of breast cancer:		
Mother	8(42.1)	-
Sister	6(31.6)	-
Daughter	4(21.0)	-
Mother & sister	1(5.3)	-
Lymph node metastases:		
Yes	23(15.3)	-
No	127(84.7)	-

Table 2. Primes selected for Exons # 1, # 4 and # 8 of Gene *ER-α*.

Polymorphi-sm site	Melting temperature (°C)	Oligonucleotic sequences	Sequence size
Exon 1	56	Primers for PCR reaction	253
	64	F 5'-GGTTTCTGAGCCTTCTGCCCTG-3'	
		R 5'-AGGCCGGTCTGACCGTAGA-3'	
Exon 4	58.5	Primers for PCR reaction	329
	65.5	F 5'-ACCTGTGTTTTCAGGGATACGA-3'	
		R 5'-GCTGCGCTTCGCATTCTTAC-3'	
Exon 8	61	Primers for PCR reaction	265
	67	F 5'-CTGTGTCTTCCCACCTACAG-3'	
		R 5'-GGGTAAAATGCAGCAGGGATT-3'	

4: 12% for exon 8) (19:1 Acrylamide: Bisacrylamide) at 200 V for 2 hours followed with 250 V for 24 hours at 16°C.

Exons 3 and 7 of the *ER-β* gene was amplified by PCR methods, set of oligonucleotide primers designed by primer3 (v. 0.4.0) soft ware (Table 3). PCR was performed for 30 cycles of 30 seconds at 95°C, 30 seconds at 58°C and 40 seconds at 72°C. Optimal electrophoretic separa-

Table 3. Primers selected for Exons #3 and #7 of Gene *ER-β.*

Polymorphi-sm site	Melting temperature (ºC)	Oligonucleotic sequences	Sequence size
Exon 3	59.23 59.86	Primers for PCR reaction F TTGCTCCCTAGAGAGACACTGA R CTTCACACGACCAGACTCCA	151
Exon 7	60.04 60.14	Primers for PCR reaction F 5' GATGAGGGGAAATGCGTAGA 3' R 5' GGCCCAGCTGTGTGATTACT 3'	156

tion for SSCP was conducted in 12% Polyacrylamide gel (29:1 Acrylamide: Bisacrylamide) at 200 V, 20 hours duration at 16°C.

After electrophoresis, the bands on gel were visualized using 0.1% silver nitrate stain. PCR samples demonstrating varying band shifting patterns as the result of first sequencing with forward primer were re-purified on agarose gel using a DNA Extraction Kit, Fermentas # K0153, Germany; then directly sequenced by big dye Terminator V3.1 Cycle Sequencing kit protocol, (Applied Biosystem Kit, Microgen Co., USA), on a sequencer ABI 3130XL (16capillaries).

We also used the PCR products purification method in order to confirm sequencing by reverse primer. The PCR products were purified using QIAquick PCR purification Kit (50) (QIAGEN cat. #28104, USA).

Ethical considerations

The study and signed informed consent were approved by the Ethics Committee of Research of the Dr. K Sharifi, The institute of Cancer, Imam Khomeini Hospital complex, Tehran University of Medical Sciences.

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

In consideration of the age at menarche at 12-years- old (and above or below) in the different gene separately and together, patients with all three polymorphisms in *ER-α* gene revealed higher, frequency, 61.9% in comparison with the normal genotype of 25.8% (*P*=0.001). However, addition of *ER-β* polymorphism in codon 392 (CTC→CTG) rs1256054, did not show additive effecting developing breast cancer (54.5%) (Table 4).

In the regards to criterion of codons together, all four codons polymorphisms (codon 10 (TCT→TCC) rs2077647, codon 352 (CCG→CCC) rs1801132and codon 594 (ACG→ACA) rs2228480, in *ER-α*, and codon 392 in *ER-β*) together, for first degree family history of breast cancer, we found out that heterozygote or homozygote polymorphisms genotype together in all four codons , with the much higher significant frequencies of 72.7% in comparison with situation of codon 392 with normal genotype (7.1%) (P= 0.001). However the results are not the same for those patients without such family history (Table 5).

For LN metastases development, when three codons in *ER-α* had variant genotypes, either heterozygote [codon 10(TCT/TCC), codon 352 (CCG/CCC) and codon 594 (ACG/ACA)] or homozygote[codon 10(TCC/TCC), codon 352 (CCC/CCC) and codon 594 (ACA/ACA)] and codon 392 had normal genotype (CTC/CTC) the distribution difference was 7.1% in comparison with when the codon of 392 in *ER-β* was also with variant genotype (CTC/CTG and CTG/CTG) with higher significant frequency of 63.6% (nine fold), and six fold higher when all codons have normal genotypes (11.8%) (Table 6).

For breast cancer, patients in the category with genotypes, combination of three SNP markers in 2, 3 or 4 as are shown in Table 7, revealed the OR< 1, especially for combination of 2 (OR 0.17, 95% CI 0.6- 0.481). Our results revealed that a combination of the three SNP markers

Table 4. Comparison of genotypic distribution frequencies in estrogen receptor-α gene (codons 10, 325, 594) with genotypic distribution frequencies in estrogen receptor- β gene (codon 392) for the age at menarche in the case group of the study population groups.

Age at menarche (years)			</=12		>12		Total		Test result
Codon 10 & 325 & 594		Codon 392	Frequency	Percent	Frequency	Percent	Frequency	Percent	
Case	Not Polymorphism[a]	Not Polymorphism[a]	24	25.8	69	74.2	93	100	χ^2=10.374
		Polymorphism[b]	4	100	-	-	4	100	*P*=0.001
		total	28	28.9	69	71.1	97	100	
	Polymorphism[b]	Not Polymorphism	26	61.9	16	38.1	42	100	χ^2=0.195
		Polymorphism	6	54.5	50	45.5	11	100	*P*=0.659
		total	32	60.4	21	39.6	53	100	
	Total	Not Polymorphism	50	37.1	85	62.9	135	100	
		Polymorphism	10	66.7	5	33.3	15	100	
		total	60	40	90	60	150	100	

[a]codon 10(TCT/TCT), codon 352 (CCG/CCG) and codon 594 (ACG/ACG), genotype; [b]heterozygote genotype codon 10(TCT→TCC), codon 352 (CCG→CCC) and codon 594 (ACG→ACA) and homozygote genotype codon 10 (TCC→TCC), codon 352 (CCGC→CCC) and codon 594 (ACA→ACA)

Table 5. Comparison of genotypic distribution frequencies in estrogen receptor-α gene (codons 10, 325, 594) with genotypic distribution frequencies in estrogen receptor- β gene (codon 392) for first-degree family history of breast cancer in the case group of the study population.

First-degree family history of breast cancer			Yes		No		Total		Test result
Codons 10 & 325& 594		Codon 392	Frequency	Percent	Frequency	Percent	Frequency	Percent	
Case	Not Polymorphism[a]	Not Polymorphism[a]	4	4.3	89	95.7	93	100	χ^2=22.250
		Polymorphism[b]	4	100	-	-	4	100	
		total	8	8.2	89	91.8	97	100	*P*=0.001
	Polymorphism[b]	Not Polymorphism	3	7.1	39	92.9	42	100	χ^2=19.627
		Polymorphism	8	72.7	3	27.3	11	100	*P*=0.001
		total	11	20.8	42	79.2	53	100	
	Total	Not Polymorphism	7	5.2	128	94.8	135	100	
		Polymorphism	12	80.0	3	20.0	15	100	
		total	19	12.7	131	87.3	150	100	

[a]codon 10(TCT/TCT), codon 352 (CCG/CCG) and codon 594 (ACG/ACG), genotype; [b]heterozygote genotype codon 10(TCT→TCC), codon 352 (CCG→CCC) and codon 594 (ACG→ACA) and homozygote genotype codon 10 (TCC→TCC), codon 352 (CCGC→CCC) and codon 594 (ACA→ACA)

Table 6. Comparison of genotypic distribution frequencies in estrogen receptor-α gene (codons 10, 325, 594) with genotypic distribution frequencies in estrogen receptor- β gene (codon 392) for lymph node metastases in the case group of study population.

	Lymph node metastases	Yes		No		Total		Test result
Codons 10&325&594	Codon 392	Frequency	Percent	Frequency	Percent	Frequency	Percent	
Normal [a]	Normal [a]	11	11.8	82	88.2	93	100	χ^2=3.275
	Not normal [b]	2	50.0	2	50.0	4	100	*P*=0.07
	Total	13	13.4	84	86.6	97	100	
Not normal [b]	Normal	3	7.1	39	92.9	42	100	χ^2=15.301
	Not normal	7	63.6	4	36.4	11	100	*P*=0.001
	Total	10	18.9	43	81.1	53	100	
Total	Normal	14	10.4	121	89.6	135	100	
	Not normal	9	60.0	6	40.0	15	100	
	Total	23	15.3	127	84.7	150	100	

[a]codon 10(TCT/TCT), codon 352 (CCG/CCG) and codon 594 (ACG/ACG), genotype; [b]heterozygote genotype codon 10(TCT→TCC), codon 352 (CCG→CCC) and codon 594 (ACG→ACA) and homozygote genotype codon 10 (TCC→TCC), codon 352 (CCGC→CCC) and codon 594 (ACA→ACA)

Table 7. Comparison of estimated selected factors with estrogen receptor-a genotypes.

Age at menarche (years)	</=12 n=60	>12 n=90	*P* value	OR (95% CI)
Breast cancer	Yes n=150	No n=147		
1	1(14.3%)	6(85.7%)	0.001	1.0(reference)
2	23(59.0%)	16(41.0%)		0.116(0.013-1.058)
3	15(55.6%)	12(44.4%)		0.133(0.014-1.264)
4	21(27.3%)	56(72.7%)		0.444(0.05-3.914)
First- degree family history of breast cancer	Affected n=19	Not affected n=131		
1	2(28.6%)	5(71.4%)	0.001	1.0(reference)
2	5(12.8%)	34(87.2%)		2.720(0.411-17.996)
3	10(37.0%)	17(63.0%)		0.68(0.111-4.182)
4	2(2.6%)	75(97.4%)		15.0(1.732-129.928)
Lymph node metastases	Yes n=23	No n=127		
1	-	7(100%)	0.033	1.0(reference)
2	5(12.8%)	34(87.2%)		1.147(1.017-1.294)
3	9(33.3%)	18(66.7%)		1.5(1.149-1.959)
4	9(11.7%)	68(88.3%)		1.132(1.044-1.228)_

Codon 10: Codon 325: Codon 594.
1) TCT/TCT:CCG/CCG:ACG/ACG;
2)TCT/TCC:CCG/CCC:ACG/ACA,TCC/TCC:CCC/CCC:ACG/ACG,TCC/TCC:CCG/CCG:ACA/ACA,TCT/TCT:CCC/CCC:ACA/ACA,CCG/CCG:ACG/ACG: ACA/ACA,TCT/TCT:CCC/CCC:ACG/ACG,TCC/TCC:CCG/CCG:ACG/ACG,TCT/TCT:CCG/CCC:ACA/ACA,TCT/TCC:CCG/CCG:ACA/ACA / TCC/TCC: CCG/CCG:ACG/ACG,TCT/TCT:CCC/CCC:ACG/ACA,TCT/TCC:CCC/CCC:ACG/ACG,TCC/TCC:CCG/CCC:ACG/ACG; 3)TCC/TCC:CCC/CCC:ACA/ACA,TCT/TCC:CCG/CCC:ACA/ACA,TCT/TCC:CCC/CCC:ACG/ACA,TCC/TCC:CCG/CCC:ACG/ACA,TCT/TCC: CCC/CCC: ACA/ACA, / TCC/TCC:CCG/CCC:ACA/ACA,TCC/TCC:CCC/CCC:ACG/ACA;
4)TCT/TCT:CCG/CCG:ACG/ACA,TCT/TCC:CCG/CCG:ACG/ACG,TCT/TCT:CCG/CCC:ACG/ACG,TCT/TCC:CCG/CCC:ACG/ACG,TCT/TCC:CCG/CCG: ACG/ACA, TCT/TCT:CCG/CCC:ACG/ACA

may decrease accuracy in predicting development of breast cancer later in their lifetime (Table 7).

For the age at menarche 12- years- old (and above or below), patients in the category with genotypes, combination of three SNP markers (2, 3 or 4), revealed the OR < 1 indicates that the condition is less likely in the age at menarche 12 or below 12 years old, especially for combination of 2 (OR 0.116, 95% CI 0.013-1.058). These results demonstrated that a combination of the three SNP markers may increase accuracy in predicting development of breast cancer for individuals with age at menarche below 12 years old.

For the first- degree family history of breast cancer, patients in the category with genotypes, combination of three SNP markers (2, 3 or 4), revealed the OR >1, especially for combination of 2 (OR 2.720, 95% CI 0.411-17.996). Our results showed that a combination of the three SNP markers may increase accuracy in predicting development of breast cancer in the first-degree relatives with history of breast cancer.

In lymph node metastases, patients in the category with genotypes, combination of three SNP markers (2, 3 or 4), revealed the greater OR than 1, especially for combination of 3 (OR 1.5, 95% CI 1.149 - 1.959). Our results demonstrated that a combination of the three SNP markers may increase accuracy in predicting LN metastases (Table 7).

Discussion

Substantial evidence has been reported that indicates that ER participates in mammary gland tumorigenesis, and thus ER is among the genes that affect breast cancer susceptibility. Breast cancer associated *ER-α* and *ER-β* polymorphisms were surveyed in previous studies [36-42]. However, there are some exceptions of no association of common genetic variations in the *ER-α* gene in relation to breast cancer risk in some studies [43, 44]. In fact, it has been

shown that *ER-α* protein over expression is common in breast cancer [27]. Somatic mutation of the *ER-α* gene has been identified [38], but *ER-α* germ-line mutation rarely occurs in the breast cancer patients. In agreement with observed low mutation rates, the present study found no novel mutations. 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-SSCP analysis of *ER-α* polymorphism.

We found when patients exhibited two polymorphisms at the same time; there were additive effects in menstruation age below12- years- old as proved risk factor in developing breast cancer. Patients individuals with the age at menarche below 12- years- old showed 53.7% polymorphisms in codon 352 and 47.6% in codon 594, but individuals with both polymorphisms (codons 325 and 594) revealed significantly increase in frequency of age at menarche below 12 years old up to 59.0%(χ^2=15.491, P=0.001). Patients with all three polymorphisms in *ER-α* gene revealed even higher significant (χ^2=14.180, P=0.001) frequency, 60.4% for the same risk factor. Nevertheless, addition of *ER-β* polymorphism in codon 392 did not show additive effect in age at menarche (54.55%). These findings together suggest that, SNP in codon 392 of estrogen receptor- β gene is more effective than those SNPs in codons 10, 325, 594, of estrogen receptor-α gene, in developing breast cancer in those patients with the first- degree family history of breast cancer. Also, SNP in codon 392 of estrogen receptor- β gene is much effective than those SNPs in codons 10, 325, 594, of estrogen receptor-α gene in developing LN metastases in breast cancer patients.

Our results demonstrated that a combination of the three SNP markers may increase accuracy in predicting LN metastases, the results matching that in the Taiwan population [35].These findings together suggest that SNP in codon 392 of estrogen receptor-β gene has more influence than SNPs in codons 10, 325, 594, of estrogen receptor-α gene in developing breast cancer in those patients with the first-degree family history of breast cancer. Also, SNP in codon 392 of estrogen receptor- β gene is more affective than those SNPs in codons 10, 325, 594 of estrogen receptor-α gene in developing LN metastases in breast cancer patients.

Conclusion

To our knowledge, this was the first systematic association study in joint effects of polymorphisms in these two genes for breast cancer risk. In current study we found that the four different common silent single nucleotide polymorphisms in estrogen receptor α and β are correlated with various aspects of breast cancer in Iran and have additive effects in increasing risk for developing breast cancer with LN metastases. Furthermore, *ER-β* variation seems to be more effective among Iranian breast cancer patients. Our data suggest that ERs polymorphisms as determined during presurgical evaluation might represent a surrogate marker for predicting breast cancer lymph node metastasis. Furthermore, our results indicate a need for more extensive evaluation of the role of other ERs polymorphism regions in susceptibility to breast cancer.

Abbreviations

BMI= Body Mass Index; CI = confidence interval; ESR = estrogen receptor; LN = lymph node; OR = odd ratio; PCR = polymerase chain reaction; Rh = Rhesus blood group system; SNP = single nucleotide polymorphism; SSCP = single-strand conformational polymorphism.

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References

[1] Screening for Breast Cancer. http://

www.who.int/cancer/detection/breastcancer/en 2007.
[2] American Cancer Society. Cancer Facts and Figures. Atlanta: American Cancer society. Last accessed July, 2011.
[3] Breast cancer facts and figures. Atlanta: American Cancer Society. Last accessed July, 2011.
[4] Mousavi SM, Montazeri A, Mohagheghi MA, Jarrahi AM, Harirchi I, Najafi M, Ebrahimi M. Breast Cancer in Iran: An Epidemiological Review. The Breast Journal 2007; 13: 383-391.
[5] Goya M. Iranian Annual Cancer Registration Report. Ministry of Health and Medical Education, Health Deputy, Center for Disease Control and Prevention. 2005 (Persian).
[6] Harirchi I, Karbakhsh M, Kashefi A, Momtahen AJ. Breast cancer in Iran: results of a multicenter study. Asian Pac J Cancer Prev 2004; 5: 24-27.
[7] Naghavi M. Mortality views in 18 Provinces of Iran. Ministry of Health, Deputy to Health Directory, Research and development office. 2003; 75 (Persian).
[8] Cady B. Breast cancer in the third millennium. Breast J 2000; 6: 280-287.
[9] WHO Model for Cancer Control, http://www.ispt.ro/supercourse/lecture/lec0701/020.htm. Accessed, 2006.
[10] Anderson E, Clarke RB, Howell A. Estrogen responsiveness and control of normal human breast proliferation. J Mammary Gland Biol Neoplasia 1998; 3: 23-35.
[11] Anderson E. The role of oestrogen and progesterone receptors in human mammary development and tumorigenesis. Breast Cancer Res 2002; 4: 197-201.
[12] Newman B, Mu H, Butler LM, Millikan RC, Moorman PG, King MC. Frequency of breast cancer attributable to BRCA1 in a population-based series of American women. JAMA 1998; 279: 915-921.
[13] Vehmanen P, Friedman LS, Eerola H, Sarantaus L, Pyrhönen S, Ponder BA, Muhonen T, Nevanlinna H. A low proportion of BRCA2 mutations in Finnish breast cancer families. Am J Hum Genet 1997; 60: 1050-1058.
[14] Whittemore AS, Gong G, Itnyre J. Prevalence and contribution of BRCA1 mutations in breast cancer and ovarian cancer: results from three U.S. population-based case-control studies of ovarian cancer. Am J Hum Genet 1997; 60: 496-504.
[15] Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, Hemminki K. Environmental and heritable factors in the causation of cancer–analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 2000; 343: 78-85.
[16] Roodi N, Bailey LR, Kao WY, Verrier CS, Yee CJ, Dupont WD, Parl FF. Estrogen receptor gene analysis in estrogen receptor-positive and receptor-negative primary breast cancer. J Natl Cancer Inst 1995; 87: 446-451.
[17] Abbasi S, Azimi C, Othman F, Daloii MRN, Ashtiani Z, Mojarrad M, Oskouei SA, Nejad FM and Ismail P. Estrogen Receptor –α Gene Codon 10 (T392C) Polymorphism in Iranian women with Breast Cancer: a case control study. Trends Mol Sciences 2009; 1: 1-10.
[18] Abbasi S, Azimi C. Genetic Polymorphisms in the Estrogen Receptor –α Gene Codon 325 (CCC/CCG) and risk of Breast Cancer Among Iranian women: a case control study. Med J Islamic Republic of Iran 2009; 23: 75-82.
[19] Abbasi S, Ismail P, Othman F, et al. Estrogen Receptor –α Gene Codon 594 (G3242A) Polymorphism among Iranian women with Breast Cancer: a case control study. Asian J Scien Res 2009; 2: 51-60.
[20] Abbasi S, Azimi C, Othman F, Einollahi N, Dashti N, Nabatchian F, Iamail P. Risk Factors for Breast Cancer in Iranian Women: A Case Control Study. Int J Cancer Res 2009; 5: 1-11.
[21] Abbasi S. Estrogen Receptor- Beta Gene Polymorphism in Women with Breast Cancer At the imam Khomeini Hospital complex, Iran. MBC Med Genetics 2010; 11: 109.
[22] Iwase H, Greenman JM, Barnes DM, Hodgson S, Bobrow L, Mathew CG. Sequence variants of the estrogen receptor (ER) gene found in breast cancer patients with ER negative and progesterone receptor positive tumors. Cancer Lett 1996; 108: 179-184.
[23] Curran JE, Lea RA, Rutherford S, Weinstein SR, Griffiths LR. Association of estrogen receptor and glucocorticoid receptor gene polymorphisms with sporadic breast cancer. Int J Cancer 2001; 95: 271-275.
[24] Vasconcelos A, Medeiros R, Veiga I, Pereira D, Carrilho S, Palmeira C, Azevedo C, Lopes CS. Analysis of estrogen receptor polymorphism in codon 325 by PCR-SSCP in breast cancer: association with lymph node metastasis. Breast J 2002; 8: 226-229.
[25] Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Ström A, Treuter E, Warner M, Gustafsson JA. Estrogen receptors: how do they signal and what are their targets. Physiol Rev 2007; 87: 905-931.
[26] Wang J, Higuchi R, Modugno F, Li J, Umblas N, Lee J, Lui LY, Ziv E, Tice JA, Cummings SR, Rhees B. Estrogen receptor alpha haplotypes and breast cancer risk in older Caucasian women. Breast Cancer Res Treat 2007; 106: 273-280.
[27] Holst F, Stahl PR, Ruiz C, Hellwinkel O, Jehan Z, Wendland M, Lebeau A, Terracciano L, Al-Kuraya K, Jänicke F, Sauter G, Simon R. Estrogen receptor alpha (ESR1) gene amplification is frequent in breast cancer. Nat Genet 2007; 39: 655-660.
[28] Iwasaki M, Hamada GS, Nishimoto IN, Netto MM, Motola J Jr, Laginha FM, Kasuga Y, Yoko-

yama S, Onuma H, Nishimura H, Kusama R, Kobayashi M, Ishihara J, Yamamoto S, Hanaoka T, Tsugane S. Isoflavine, polymorphisms in estrogen receptor genes and breast cancer risk in case-control studies in Japanese, Japanese Brazilians and non- Japanese Brazilians. Official J Japan cancer Assoc 2009; 100: 927-933.
[29] Surekha D, Sailaja K, Nageswara Rao D, Raghunadharao D, Vishnupriya S. Oestrogen receptor beta (ER-β) polymorphism and its influence on breast cancer risk. G Gent 2009; 88: 261-266.
[30] Srinivasan V, Spence DW, Pandi-Perumal SR, Trakht I, Esquifino AI, Cardinali DP, Maestroni GJ. Melatonin, environmental light, and breast cancer. Breast cancer research and treatment 2008; 108: 339-350.
[31] Fuqua SA, Wiltschke C, Zhang QX, Borg A, Castles CG, Friedrichs WE, Hopp T, Hilsenbeck S, Mohsin S, O'Connell P, Allred DC. A hypersensitive estrogen receptor-α mutation in premalignant breast lesions. Cancer Res 2000; 60: 4026-4029.
[32] Zubairy S, Cui Y, Fuqua SA. The K303R estrogen receptor alpha breast cancer mutant generates a new Akt kinase site. Proc Am Assoc Cancer Res 2004; 45: 659.
[33] Rouzier R, Perou CM, Symmans WF, Ibrahim N, Cristofanilli M, Anderson K, Hess KR, Stec J, Ayers M, Wagner P, Morandi P, Fan C, Rabiul I, Ross JS, Hortobagyi GN, Pusztai L. Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 2005; 11: 5678-5685.
[34] Huang E, Cheng SH, Dressman H, Pittman J, Tsou MH, Horng CF, Bild A, Iversen ES, Liao M, Chen CM, West M, Nevins JR, Huang AT. Gene expression predictors of breast cancer outcomes. Lancet 2003; 361: 1590-1596.
[35] Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 2006; 295: 2492-2502.
[36] Oh DS, Troester MA, Usary J, Hu Z, He X, Fan C, Wu J, Carey LA, Perou CM. Estrogen-regulated genes predict survival in hormone receptor-positive breast cancers. J Clin Oncol 2006; 24: 1656-1664.
[37] Hsiao WC, Young KC, Lin SL, Lin PW. Estrogen receptor-α polymorphism in a Taiwanese clinical breast cancer population: a case–control study. Breast Cancer Res 2004; 6: R180-R186.
[38] Southey MC, Batten LE, McCredie MR, Giles GG, Dite G, Hopper JL, Venter DJ. Estrogen receptor polymorphism at codon 325 and risk of breast cancer in women before age forty. J Natl Cancer Inst 1998; 90: 532-536.
[39] Kang HJ, Kim SW, Kim HJ, Ahn SJ, Bae JY, Park SK, Kang D, Hirvonen A, Choe KJ, Noh DY. Polymorphisms in the estrogen receptor-alpha gene and breast cancer risk. Cancer Lett 2002; 178: 175-180.
[40] Murphy LC, Dotzlaw H, Leygue E, Douglas D, Coutts A, Watson PH. Estrogen receptor variants and mutations. J Steroid Biochem Mol Biol 1997; 62: 363-372.
[41] Yu KD, Rao NY, Chen AX, Fan L, Yang C, Shao ZM. A systematic review of the relationship between polymorphic sites in the estrogen receptor-beta (ESR2) gene and breast cancer risk. Breast Cancer Res Treat 2011; 126: 37-45.
[42] Sakoda LC, Blackston CR, Doherty JA, Ray RM, Lin MG, Gao DL, Stalsberg H, Feng Z, Thomas DB, Chen C. Selected estrogen receptor 1 and androgen receptor gene polymorphisms in relation to risk of breast cancer and fibrocystic breast conditions among Chinese women. Cancer Epidemiol 2011; 35: 48-55.
[43] Einarsdóttir K, Darabi H, Li Y, Low YL, Li YQ, Bonnard C, Sjölander A, Czene K, Wedrén S, Liu ET, Hall P, Humphreys K, Liu J. ESR1 and EGF genetic variation in relation to breast cancer risk and survival. Breast Cancer Res 2008; 10: R15.
[44] Slattery ML, Sweeney C, Herrick J, Wolff R, Baumgartner K, Giuliano A, Byers T. ESR1, AR, body size, and breast cancer risk in Hispanic and non-Hispanic white women living in the Southwestern United States. Breast Cancer Res Treat 2007; 105: 327-335.
[45] Gonzalez-Zuloeta Ladd AM, Vásquez AA, Rivadeneira F, Siemes C, Hofman A, Stricker BH, Pols HA, Uitterlinden AG, van Duijn CM. Estrogen receptor α polymorphisms and postmenopausal breast cancer risk. Breast Cancer Research and Treatment 2008; 107: 415-419.