

## Estrogen Receptor Alpha Gene Single Nucleotide Polymorphisms, +2464 C/T and -4576A/C, and Breast Cancer Risk, a Hospital-Based Case-Control Study

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### ABSTRACT

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**Background:** Estrogen is a risk factor for the development of breast cancer. The effect of estrogen is primarily mediated by estrogen receptor alpha 1 (ESR1). In this study, we investigated the association between breast cancer risk and the frequency of alleles and genotypes for two ESR1 single nucleotide polymorphisms (SNPs) in breast cancer patients and a healthy control group.

**Methods:** A total of 98 female patients with pathologically confirmed breast cancer and 93 age-matched healthy female controls who were selected from the visitors of the general hospital were recruited in the study. Two ESR1 candidate polymorphisms; +2464 C/T (rs3020314) and -4576 A/C (rs1514348) were selected. The frequency of alleles and genotypes was determined using Quantitative Real-Time PCR assay. Linkage disequilibrium (LD) was assessed for each pair of markers. Using logistic regression, genotype frequencies were estimated as odds ratios with 95% confidence intervals.

**Results:** There was no significant difference in the genotype and allele distributions of ESR1 for SNPs +2464 C/T and SNP -4576 A/C between patients and controls. The frequency of the ESR1 +2464 T/T genotype in case and control groups was 31.6% vs 29.0%, (OR<sub>TT/TC</sub>: 1.13, 95%CI: 0.58, 2.20; P = 0.69). The frequency of the +2464C allele was 33.9% vs 35.2%, (OR<sub>C/T</sub>: 0.94, 95%CI: 0.60, 1.47; P = 0.79). The frequency of the ESR1 -4576C/C genotype in case and control groups was 37.75% vs 33.36%, OR<sub>CC/AC</sub>: 1.02, 95%CI: 0.51, 1.97; P = 0.98). The frequency of the -4576A allele was 36.2% vs 43.6 %, (OR<sub>C/A</sub>: 0.73, 95%CI: 0.47, 1.13; P = 0.14).

**Conclusion:** The results indicated that ESR1 polymorphism does not show any significant association with breast cancer risk among female Iranian adults.

**Keywords:** Estradiol receptor, Single nucleotide polymorphism, Breast neoplasm, Association study.

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## INTRODUCTION:

**B**reast cancer is the most common cause of cancer-related death among women worldwide<sup>1</sup>. There is a growing prevalence of breast cancer in developing countries<sup>2</sup>. Recent reports indicate that breast cancer was the most prevalence cancer among Iranian women in 2012<sup>3</sup>. The molecular mechanism that induces or develops breast cancer has not yet been fully understood, but genetic factors have been shown to have significant effects on the development of breast cancer<sup>4,5</sup>. In addition, environmental, occupational and even infectious agents were suggested to be involved in the occurrence of this disease<sup>6,7</sup>.

Estrogen is responsible for the growth, development, and regulation of the female reproductive system<sup>8</sup>. The actions of estrogen are mediated by the estrogen receptor (ER), a nuclear protein that binds to DNA and controls gene expression<sup>9</sup>. There are two main forms of ER, estrogen receptor alpha (ER- $\alpha$ ) and ER- $\beta$ . ER- $\alpha$  and ER- $\beta$  are encoded by distinct genes, *ESR1* and *ESR2* which are located on different chromosomes<sup>9</sup>. The human *ESR1* gene is located on chromosome 6 while the *ESR2* gene is on chromosome 14<sup>10,11</sup>. *ESR1* gene plays a crucial role in the development and pathogenesis of breast cancer<sup>12-14</sup>. The effect of estrogen on the breast epithelium is mainly mediated by estrogen receptor alpha<sup>15</sup>. Several studies have shown associations between *ESR1*/polymorphisms and breast cancer risk<sup>16-18</sup>, whereas other studies did not find any association between *ESR1* variants and breast cancer risk in numerous populations<sup>19-21</sup>. Therefore, the role of *ESR1*/polymorphisms in breast cancer risk remains unclear. In a hospital based case/control study of 360 breast cancer cases and 672 controls in Norway, the authors reported significant association between the A allele of rs9340799 and breast cancer risk<sup>22</sup>. In another study, the association between the same SNP, *ESR1* rs9340799, and breast cancer risk was evaluated in 205 breast cancer cases

and 205 age-matched controls in Korean women, but they reported only a significant decrease of breast cancer risk with the G allele (OR = 0.4; 95% CI: 0.3–0.6)<sup>23</sup>. Nevertheless, in a large Swedish study including 1557 cases and 1512 controls, no association was observed between rs9340799 SNP and breast cancer risk<sup>24</sup>.

The aim of this study was to assess the frequency of two variants of *ESR1*; rs3020314 and rs1514348 and their possible associations with breast cancer risk in a hospital based case-control study. The selection of these two SNPs was based on a couple of studies including the report of Lipphardt et al. who identified a significant association between *ESR1* intron-4576A/C (rs1514348), breast cancer susceptibility and progesterone receptor status in the central European Caucasian population<sup>25</sup>. In another large study with 4,470 cases and 4,560 controls, Mavaddat et al. showed that *ESR1* intron +2464 C/T (rs3020314) has an overall effect on breast cancer risk and estrogen receptor status among patients in eastern European countries<sup>26</sup>.

## METHODS:

### Study population

The study population was recruited from the clinics of the Cancer Institute of Iran, Tehran University of Medical Sciences, Tehran, Iran between October 2015 and March 2016. The cases consisted of 98 female breast cancer patients with histopathologically confirmed malignant tumors and the control group included 93 healthy female individuals. Patients with a history of autoimmune diseases and those suffering from diseases such as diabetes mellitus or thyroid disorders were excluded from the study. The control group consisted of healthy women who were visiting a general hospital (i.e. Imam Khomeini hospital), where the Cancer Institute of Iran is located. These individuals were visiting non-cancer patients at Imam Khomeini hospital. The controls were selected from the same region in order to represent the target population (Iranian women). This project was approved by the Ethical Committee of Tehran University

of Medical Sciences, and written informed consent was obtained from all cases and controls.

#### DNA Extraction and Genotyping Analysis:

Genomic DNA was extracted from peripheral blood leukocytes using the proteinase K phenol-chloroform extraction procedure<sup>27</sup>. DNA purity and concentrations were determined by spectrophotometric measurement of absorbance at 260 and 280 nm using a UV spectrophotometer. The extracted DNA was stored at  $-20^{\circ}\text{C}$  until analysis.

The selected two *ESR1* SNPs [+2464 C/T (rs3020314) and -4576A/C (rs1514348)] were genotyped using a TaqMan real-time PCR allelic discrimination assay. An ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, USA) was used in accordance with the instructions provided by the manufacturer. The allelic discrimination was determined using ABI SDS V 1.4 software.

#### Statistical Analysis

Statistical analysis of genotype frequencies in association with breast cancer risk was performed using the Pearson's  $\chi^2$  tests, on the basis of deviations of genotype frequencies in controls. These deviations were calculated with the Hardy-Weinberg formula. Using logistic regression, genotype risks were estimated as odds ratios with 95 % confidence intervals. The most prevalent SNP was considered as the reference group (wild type). Stata version 14 (STATA Corps, College Station, TX, USA) was used for all analyses.

#### RESULTS:

Breast cancer patients and controls in this study were all female, and were predominantly middle-aged at the time of diagnosis (Mean age:  $53.51 \pm 11.21$  in cases and  $48.06 \pm 11.73$  in controls). The demographic and clinical characteristics of the study subjects are presented in **Table 1**. The distribution of genotypes for the two poly-

**Table 1. Demographic and Clinical Characteristics of Breast Cancer Cases and Controls**

Variables	Controls (N = 98)	Patients (N = 93)	P-value
Age (years)	48.06 $\pm$ 11.73	53.51 $\pm$ 11.21	0.001
Age at First Pregnancy (years)	19.70 $\pm$ 4.45	20.26 $\pm$ 4.93	0.410
BMI (kg/m <sup>2</sup> ) <sup>a</sup>	28.28 $\pm$ 4.87	28.13 $\pm$ 4.60	0.827
<b>Marital Status</b>			
Single	10 (10.75%)	6 (6.12%)	0.506
Married	72 (77.42%)	79 (80.61%)	
Divorced /Widowed	11 (11.83%)	13 (13.27%)	
<b>Education Level</b>			
Illiterate	20 (21.74%)	23 (23.71)	0.944
$\leq 12$ years of education	55 (59.78%)	56 (57.73%)	
$> 12$ years of education	17 (18.48%)	18 (18.56%)	
<b>Family History of Breast Cancer<sup>b</sup></b>			
Yes	7 (7.61%)	16 (18.18%)	0.034
No	85 (92.39%)	72 (81.82%)	
<b>Age at Menarche</b>			
$< 12$ years	7 (7.69%)	0	$< 0.0001$
12-15 years	77 (84.62%)	97 (98.98%)	
$> 15$ years	7 (7.69%)	1 (1.02%)	
<b>Menopause Status</b>			
Yes	35 (37.63%)	49 (50.52%)	0.074
No	58 (62.37%)	48 (49.48%)	

a BMI, body mass index; b Family history is defined as having at least one first degree relative with breast cancer.

**Table 2. Allele and genotype frequencies of *ESR1* SNPs [+2464 C/T (rs3020314) and -4576A/C (rs1514348)] Polymorphisms among women with breast cancer and healthy female individuals.**

Polymorphism	Cases(98)	Controls (93)	OR (CI95%)	P-value
<b>+2464 C/T (rs3020314)</b>				
TC	65 (66.3)	64 (68.8)	1	-
TT	31 (31.6)	27 (29)	1.13 (0.58, 2.20)	0.698
CC	2 (2.1)	2 (2.2)	0.98 (0.06, 13.96)	0.987
T allele	127 (66.1)	118 (64.8)	1	0.789
C allele	65 (33.9)	64 (35.2)	0.94 (0.60, 1.47)	
<b>-4576 A/C (rs151434)</b>				
AC	51(52.04)	43 (46.21)	1	-
CC	37 (37.75)	31 (33.36)	1.02 (0.51, 1.97)	0.984
AA	10 (10.20)	19 (20.43)	0.44 (0.16, 1.13)	0.062
C allele	125 (63.8)	105 (56.4)	1	-
A allele	71 (36.2)	81 (43.6)	0.73(0.47, 1.13)	0.143

morphisms studied were in Hardy-Weinberg Equilibrium in both patients and healthy controls ( $P > 0.05$ ). The genotype and allele frequencies for the *ESR1* SNPs [+2464 C/T (rs3020314) and -4576A/C (rs1514348)] are shown in **Table 2**.

The frequency of the *ESR1* +2464 T/T genotype in case and control groups was 31.6% vs 29.0%, ( $OR_{TT/TC}$ : 1.13, 95%CI: 0.58, 2.20;  $P = 0.69$ ). The frequency of the +2464C allele was 33.9% vs 35.2 %, ( $OR_{C/T}$ : 0.94, 95%CI: 0.60, 1.47;  $P = 0.79$ ). The frequency of the *ESR1* -4576C/C genotype in case and control groups was 37.75% vs 33.36 %,  $OR_{CC/AC}$ : 1.02, 95%CI: 0.51, 1.97;  $P = 0.98$ ). The frequency of the -4576A allele was 36.2% vs 43.6 %, ( $OR_{C/A}$ : 0.73, 95%CI: 0.47, 1.13;  $P = 0.14$ ). There was no significant difference in genotype and allele distributions of the *ESR1* for SNPs +2464 C/T (rs3020314) and SNP -4576A/C (rs1514348) between patients and controls ( $P > 0.05$ ).

## DISCUSSION:

In this hospital based case-control study we found no overall association between two *ESR1* single nucleotide polymorphisms and risk of breast cancer. Several groups have studied the relation between *ESR1* poly-

morphisms and breast cancer risk, but the results have been inconsistent<sup>18-21</sup>. In the present report, we investigated the association between two *ESR1* SNPs; +2464 C/T and -4576A/C, and breast cancer risk. We did not observe a significant association between *ESR1* +2464 or -4576A/C polymorphisms and breast cancer risk.

The results of the present study are consistent with some previous reports<sup>19-21</sup>. In a Korean study population including 155 women, 110 with breast cancer and 45 without cancer, Kang et al. did not find any significant association between five of the known polymorphisms of *ESR1* gene and breast cancer risk<sup>28</sup>. In another case-control study, involving 412 African-American and Caucasian women in the United States (220 cases and 192 controls with equal distribution between the two ethnic groups), the associations between *ESR1* polymorphisms, bone density and breast cancer susceptibility were investigated. They did not find any association between *ESR1* genotypes and breast cancer risk<sup>29</sup>. Furthermore, in a large case-control study of 1,069 Chinese women with breast cancer and 1,169 age-matched controls, Cai and colleagues did not observe a significant difference between patients and

controls in the distribution of the *ESR1* rs9340799 polymorphism. Their results showed only a non-significant elevated level of A allele risk in post-menopausal woman<sup>30</sup>.

Contrasting with our findings, Dunning et al. conducted studies on more than 55,000 breast cancer cases and controls in the European population to evaluate genetic variation in *ESR1* and breast cancer risk. They found that *ESR1*; +2464 C/T (rs3020314) has an overall effect on breast cancer characteristics, including histology, staging and grading of tumor<sup>31,32</sup>. These authors also observed higher susceptibility to breast cancer among individuals with the CT genotype compared to individuals with the TT genotype<sup>32</sup>.

The main limitation of our study was the sample size which did not have enough power to detect associations between further genotypes of the *ESR1* gene and the risk of disease. Moreover, having detailed data on the participants could help to adjust the association and may lead us to explore correlations in subgroup analyses. In conclusion, our results showed no differences in allele or genotype frequencies of the *ESR1*; +2464 C/T (rs3020314) and the *ESR1*; -4576A/C (rs1514348) polymorphisms between breast cancer cases and the respective control group. The results showed no association between *ESR1* polymorphism and breast cancer risk in our female population. We suggest further studies with a larger sample size among the Iranian population.

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