

Study of Polymorphism of the Estrogen Receptor Alpha Gene as a Genetic Marker for the Risk of Breast Cancer

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Abstract— The aim of this study is to find the correlation between genetic polymorphisms in the estrogen receptor alpha gene and risk of breast cancer in a trial to be taken as a genetic marker for the early detection of breast cancer. **Methods:** 70 women attending the Oncology Center and General Surgery Departments were included and divided into 3 groups: Group A: 25 with breast cancer. Group B: 20 with benign breast tumors. Group C: 25 free from any malignancy or breast masses and were served as a control group. All patients and controls were subjected to full history taking and complete clinical examination. Blood samples withdrawn from all women were used for DNA isolation and detection of polymorphisms of estrogen receptor alpha gene by polymerase chain reaction and restriction enzymes. Tissue sections from breast tumors were used for immunohistochemistry staining of Estrogen receptors and Progesterone receptors. **Results:** The polymorphism at the PvuII restriction site (p allele) was associated with significant increase in the risk of breast cancer. The XbaII polymorphism was associated with a non-significant increased risk for breast cancer. There is a significant decrease in the age of menarche of individuals with presence of PvuII and XbaI restriction sites compared with individuals with absence of these restriction sites. **Conclusions:** The combined analysis of multiple parameters in this study supported the presence of genetic polymorphisms that are associated with the risk of breast cancer. This may help to assess the risk individual more precisely for early detection of breast cancer and to select the candidates for hormonal and chemoprevention more efficiently.

Keywords— Breast cancer, Estrogen receptor alpha, Genetic marker, Polymorphism.

I. INTRODUCTION

BREAST cancer is the most common cancer in women [1] and it is one of the leading causes of cancer-related deaths for women [2]. Breast cancer is a heterogeneous disease that is associated with genetic and environmental factors [3]. Genome-wide association studies (GWAS) identified several genetic risk factors for breast cancer [4]. Prior to genetic studies, investigations have mainly revolved around the risk

factors associated with breast cancer that can be grouped into three broad determinants: family history (hereditary) factors, hormonal and reproductive-related risk factors, and environmental (including lifestyle) factors [3],[5].

Estradiol binds with high affinity to estrogen receptor alpha. This binding induces DNA synthesis, cell division, and production of growth factors and progesterone receptor proteins. Estrogens and progesterone are essential for normal mammary gland development and function, but their stimulation of breast cell proliferation may be procarcinogenic. Many of the identified risk factors for breast cancer can be explained by their effects on lifetime exposure to estrogen and other hormones [6]. In postmenopausal women with breast cancer, the tumor concentration of estradiol is high, because of in situ aromatization, despite the presence of low serum estradiol concentrations [7]. Although estrogens are important physiological regulators in the reproductive system, in bone metabolism, and in the maintenance of the cardiovascular and central nervous systems, they have also been associated pathologically with an increased risk for breast and endometrial cancer [8],[9].

Estrogen receptors (ERs) are members of the nuclear receptor (NR) family that mediate the pleiotropic effects of the steroid hormone estrogen in a diverse range of developmental and physiological processes [10]. ERs belong to a family of transcription factors, the nuclear receptor super family, responsible for mediating the effects of steroids on development, reproduction, proliferation, cellular homeostasis and gene expression [11]. The estrogen receptor alpha (ER α) is one of the most important mediators of hormonal response in estrogen-sensitive tissues such as the breast [12] and plays a crucial role in breast growth and differentiation as well as in the development of cancer [13]. The human ER α gene is localized on chromosome 6q24-q27 [14],[15], it extends more than 140 kb and includes eight exons [16].

Because ER α is an important mediator of the hormonal response in estrogen sensitive tissues, the genetic polymorphisms on the ER were therefore postulated as the potential risk factors of breast cancer [17]. Several ER α gene polymorphisms have been reported, among which PvuII and XbaI polymorphisms are the most studied in several diseases, including breast cancer [18]-[20].

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Some polymorphisms in the genes coding for these receptors may change the expression of the receptors and may, therefore, modify the effect of hormone on mammographic density [21] and hence breast cancer. However, Ladd et al. [20] suggested that the ER α polymorphisms do not play a role in breast cancer risk in Caucasian postmenopausal women. So, data and conclusions are inconsistent and controversial [2].

Aim of the work: the aim of this study is to find if there is a correlation between genetic polymorphisms in the estrogen receptor alpha (ER α) gene and risk of breast cancer in Egyptian females in a trial to be taken as a genetic marker for early detection of breast cancer. Hopefully, this finding will help to select early candidates of breast cancer for hormonal and chemoprevention.

II. SUBJECTS AND METHODS

A. Subjects

A total of 70 Egyptian women attending the Oncology Center and General Surgery Departments in Mansoura University Hospital were included. The ages of all women were ranged from 19 to 65 years.

The selected women were divided into 3 groups: Group I: 25 women attended Oncology Center, Mansoura University with breast cancer were included in this study as breast cancer cases. Diagnosis was based on histopathological examination of tissue biopsy from breast masses. *Group II:* 20 women with benign breast tumors. *Group III:* 25 women who were clinically free from any malignancy or breast masses and age matched with cases and they served as a control group. This group was selected from women attended General Surgery Departments, Mansoura University Hospital to undergo minor surgical operations unrelated to Oncology or Endocrinology.

B. All patients and controls were subjected to

Full history taking. Complete clinical examination and all participants were measured regarding weight, heights and BMI. A structured questionnaire was used to elicit detailed information on age, menstrual and reproductive histories, prior breast disease history, hormone use, smoking, and alcohol drinking.

Written informed consent was obtained from all cases and control volunteers according to the declaration of Helsinki and the study was approved by the Ethics Committee of College of Medicine, Mansoura university.

Exclusion criteria

The following patients were excluded from our study: Patient with recurrent breast cancer. Patient receiving chemotherapy or radiotherapy. Patients with present or past history of any malignancy. Patients with present or past history of endocrine disorders.

C. Samples:

I) One ml random blood samples were withdrawn from all women in the three groups, collected into tubes containing

EDTA as an anticoagulant stored at -20°C until used for DNA extraction and utilized for detection of some polymorphisms of estrogen receptor alpha gene by polymerase chain reaction (PCR) and restriction enzymes.

2) Tissue sections from breast tumors for immunohistochemistry staining of: Estrogen receptors and Progesterone receptors.

D. Methodology

DNA Extraction: Genomic DNA was extracted from buffy coat fractions using the Puregene DNA Isolation kit (Gentra Systems, Minneapolis, MN) following the manufacturer's protocol. DNA concentration was measured by PicoGreen dsDNA Quantitation kit (Molecular Probes, Eugene, OR). Ten ng of genomic DNA were used for each PCR. ER- genotypes were determined with a PCR-RFLP method. The primers for analysis were: 5'-CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3' (forward) and 5'-TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3' (reverse). These primers generated a 1.3-kb fragment. The PCR product contains a part of intron 1 and exon 2 of the ER- α gene. The PCR products were digested by the PvuII and XbaI restriction endonucleases, respectively. The DNA fragments were then separated using 1.5% agarose gel and detected by ethidium bromide staining. PP and XX, signifying the absence of restriction sites, gave one 1.3-kb fragment. Pp, signifying the presence of PvuII restriction sites on both alleles, was digested into two fragments (0.85 and 0.45 kb). The xx genotype was revealed by XbaI digestion into two fragments (0.9 and 0.4 kb). Genotyping data were obtained from cases and controls who have blood samples, which represent eligible case patients and eligible control subjects.

III. STATISTICAL ANALYSIS

The statistics were used to evaluate case - control difference in the distribution of allele types and genotypes. All data were analyzed using statistical package for social science program (SPSS). The results were expressed as the mean \pm SD. Measurement data were analyzed using one-way analysis of variances (ANOVA). When the distribution of data was nonparametric, Mann-Whitney (unpaired Wilcoxon) test was used for comparison between two groups. Spearman rank and Pearson correlation coefficient were used to find the degree of correlation between the different variables. P value ≤ 0.05 was considered significant.

IV. RESULTS

The results obtained from present study can be summarized as follow:

The control, benign and cancer groups were comparable in age, there was statistically insignificant difference in age in both benign group and cancer group compared with control group. It was found that the polymorphism at the PvuII restriction site (p allele) was associated with statistically

significant increase in the risk of breast cancer *table (1)*. While, The XbaII polymorphism was non-significantly associated with an increased risk for breast cancer *table (2)*.

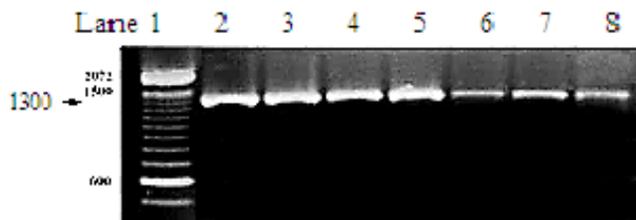


Fig. 1 Agarose gel showed amplified PCR products of ER- α gene. Lanes from 2 to 8 showed amplified PCR products and lane 1 showed DNA marker. The arrow on the left indicates molecular weight of PCR products (1.3 Kb).

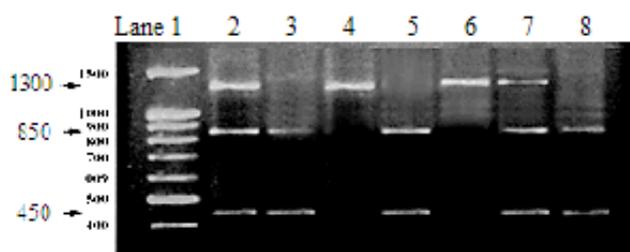


Fig. 2 Agarose gel showed digested products of ER- α gene by PvuII restriction enzyme. Each sample revealed one of three electrophoretic patterns as in figure: PP genotype indicating absence of PvuII restriction site from both alleles giving one band at 1.3 Kb (lanes 4 and 6). pp genotype indicating the presence of PvuII restriction sites on both alleles, giving two bands at 0.85 and 0.45 kb (lanes 3,5 and 8). Pp genotype indicating the presence of PvuII restriction sites on one of the two alleles, and the other allele does not contain restriction sites, and gave one 1.3 kb fragment. So Pp gave three bands at 1.3, 0.85 and 0.45 kb (lanes 2 and 7). Lane 1 showed DNA marker.

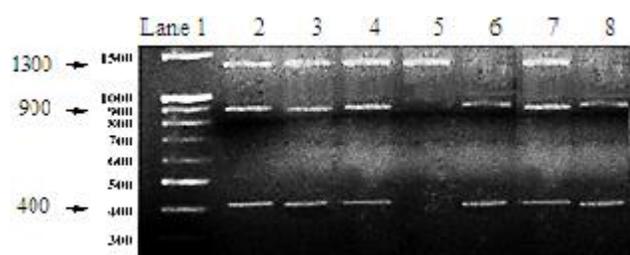


Fig. 3 Agarose gel showed digested products of ER- α gene by XbaI restriction enzyme. Each sample revealed one of three electrophoretic patterns: XX genotype indicating absence of XbaI restriction site from both alleles giving one band at 1.3 Kb (lane 5). xx genotype indicating the presence of XbaI restriction sites on both alleles, giving two bands at 0.9 and 0.4 kb (lanes 6 and 8). Xx genotype indicating the presence of XbaI restriction sites on one of the two alleles, and the other allele does not contain restriction sites, and gave one 1.3 kb fragment. So Xx gave three bands at 1.3, 0.9 and 0.4 kb. Lane 1 showed DNA marker.

Table (1) showed frequencies of ER- α gene PvuII polymorphisms in control and cancer groups. The P allele was more prevalent among controls (56%) than cases (40%) in PvuII polymorphism. About 44% of control and 16% of cases were homozygous and 24% of control and 48% of cases are heterozygous for this allele. The presence of p allele increases risk of cancer breast ($P = 0.031$). Odd ratios (ORs) for genotypes Pp and pp were 5.5 (95% CI, 0.9 – 13.9) and 3.9 (95% CI, 0.6 – 8.2) respectively, comparing to genotype PP.

TABLE I
POLYMORPHISMS OF ER-A PVUII AND RISK OF CANCER BREAST

Variable	Control Group	Cancer Group	OR	95% CI
PP	11 44%	4 16%	1.0	Ref.
Pp/pp	14 56%	21 84%	3.4	0.7 – 6.8
Pp	6 24%	12 48.0%	5.5	0.9 – 13.9
pp	8 32%	9 36%	3.9	0.6 – 8.2
$P =$	0.031			

The frequencies of ER- α gene PvuII polymorphisms in control and breast benign tumor groups: There was statistically insignificant changes in the the P allele distribution among controls (56%) and benign (50%) in PvuII polymorphism. About 44% of control and 30% of benign were homozygous and 24% of control and 40% of benign are heterozygous for this allele.

Table (2) showed frequencies of ER- α gene XbaI polymorphisms in control and cancer groups. There was statistically insignificant changes in the the X allele distribution among controls (44%) and cancer cases (30%) in XbaI polymorphism. About 24% of control and 8% of cancer were homozygous and 40% of control and 44% of cancer are heterozygous for this allele. Odd ratios (ORs) for genotypes Xx and xx were 3.3 (95% CI, 0.4 – 6.9) and 4.0 (95% CI, 0.5 – 8.8) respectively, comparing to genotype XX.

TABLE II
POLYMORPHISMS OF ER-A XBAI AND RISK CANCER BREAST

Variable	Control Group	Cancer Group	OR	95% CI
XX	6 24%	2 8%	1.0	Ref.
Xx/xx	19 76%	23 92%	3.6	0.6 – 9.7
Xx	10 40%	11 44%	3.3	0.4 – 6.9
xx	9 36%	12 48%	4.0	0.5 – 8.8
$P =$	0.123			

The frequencies of ER- α gene XbaI polymorphisms in control and breast benign tumor groups: There was statistically insignificant changes in the the X allele distribution among controls (44%) and benign (45%) in XbaI polymorphism. About 24% of control and 20% of benign were homozygous and 40% of control and 50% of benign are heterozygous for this allele.

TABLE III
RELATIONSHIP BETWEEN POLYMORPHISMS OF BOTH ER-A PvuII & ER-A XbaI AND AGE OF MENARCHE

Variable	Number	Mean age (year)	± S.D	P
PP	21	13.9	1.6	0.021
Pp/pp	49	12.9	1.6	
XX	12	14.8	1.5	< 0.001
Xx/xx	58	12.9	1.5	

TABLE IV
GENOTYPE COMBINATIONS OF PvuII AND XbaI POLYMORPHISMS AND RISK OF CANCER BREAST

Genotype	Control Group	Cancer Group	OR	95% CI
PPXX	4 16%	2 8%	1	Ref
ppxx	6 24%	7 28%	2.3	0.2 – 3.9
Ppxx	2 8%	4 16%	4	0.1 – 4.5
PPxx	1 4%	1 4%	2	0.1 – 3.9
ppXx	2 8%	2 8%	2	0.4 – 4.1
PpXx	2 8%	8 32%	8	0.8 – 13
PPXx	6 24%	1 4%	0.3	0.1 – 8
PpXX	2 8%	0 0%	0	

Genotype combinations of both PvuII and XbaI polymorphisms and risk of cancer breast: The genotypes containing one or two p or x alleles were, in general, associated with an elevated risk of cancer breast and the highest OR was observed for the genotype with Pp and Xx (OR = 8, CI, 0.8 - 13) table (4).

V. DISCUSSION

In recent years, the association of genetic susceptibility to cancers has drawn more and more attention to the study of polymorphisms of genes involved in tumorigenesis and other diseases [2]. Similar to other cancer types, genetic factors play a central role in the development and progression of breast cancer [22]. Studies showed that excessive estrogen from the exogenous source can have pathological consequences in human cell, and result in the alteration of tumors, including the occurrence of breast cancer [23]. The ER α gene encodes a transcription factor with an estrogen-binding domain, an activation domain, and an estrogen response element (ERE) DNA-binding domain. By regulating the cell proliferation and differentiation via paracrine mechanism, ER α is believed to be tightly associated with breast cancer [24]. Therefore, genetic variations in the ER α gene, which can lead to disordered estrogen activity, become a potential risk for breast cancer.

Single-nucleotide polymorphisms (SNPs) of ER α have been studied in numerous clinical studies. Many association studies on this gene have been confined to 2 SNPs (originally detected with the restriction enzymes PvuII and XbaI [25], which are located in the first intron of ER α . The ER α PvuII and XbaI

polymorphisms have been associated to tumorigenesis and many other diseases [26], involving heterogeneous conclusions. Li and Xu reported that ER α PvuII (C>T) polymorphism placed pre-menopausal women at risk for breast cancer, but XbaI (A>G) polymorphism is not associated with the risk of breast cancer [27]. P325P polymorphism in the exon 4 of ER α gene has been found to be associated with bone mineral density in post-menopausal women [28]. Korean women carrying both the ER α P325P CC and CDK7 Ex2-28C>T (rs2972388) TT genotypes have been shown to be at increased breast cancer risk [29]. However, because of the heterogeneous of data sources and analysis methods, the conclusions in many of these studies were inconsistent and controversial. The association of genetic polymorphisms in the ER α and the risk of breast cancer have been of increasing interest [30].

Most of the studies in ER α gene polymorphisms and breast cancer were conducted in the western countries. Since Egyptian women may have different genotype distributional different level of susceptibility compared with western women, we conducted a hospital based case control study to examine this issue further by evaluating the potential association between genetic polymorphism of intron 1 (PvuII and XbaI) of ER α gene and breast cancer risk in Egyptian women.

In this case control study we investigated PvuII and XbaI polymorphisms in cancer breast. It was found that the polymorphism at the PvuII restriction site (p allele) was associated with statistically significant increase in the risk of breast cancer ($P = 0.031$). The XbaI polymorphism was associated with a non-significant increased risk for breast cancer ($P > 0.05$).

Possible explanations of How breast cancer risk is affected by the intronic PvuII polymorphism of the ER α gene include: (a) the intronic polymorphism may be in linkage disequilibrium with exon alteration, which affects ER protein function; (b) the PvuII polymorphism in the ER α gene may be linked with the alteration of another unidentified gene adjacent to the ER α gene, which increases breast cancer risk [31]; (c) intronic changes in gene sequence may have an impact on the expression of other genes by influencing the transcription and/or stability of mRNA of those genes [32],[33]; (d) and some introns contain regulatory sequences such as enhancers, which affect the levels of expression through transcriptional regulation [34],[35].

Two studies have suggested that the ER α PvuII P allele increases ER α transcription [36], whereas another study suggested that the PvuII p allele and the XbaI x allele increases transcription of ER α . These results indicate that PvuII and XbaI polymorphisms are involved in the production of ER α , but the exact function needs to be clarified [21]. However, it has been frequently reported that the PvuII and XbaI polymorphisms of the ER α gene are related to breast cancer [37],[38]. The results of our study are in agreement with Onland-Moret et al. [39] who found an increased breast cancer risk related to the PvuII polymorphism p allele, and a

nonsignificantly elevated risk for the XbaI polymorphism x allele. Also, Cai et al. [40] demonstrated increased risk of cancer breast associated with PvuII polymorphism (p allele) and non significant increased risk of cancer breast with XbaI polymorphism. Also, the results of Shen et al. [17] indicated that both ER α PvuII Pp/pp and ER α XbaI Xx/xx genotypes may increase the risk of breast cancer. However, contradictory to our results in a study of 360 breast cancer patients from a hospital in Norway and 672 convenient controls, Andersen et al. [18] found that allele frequencies of the PvuII polymorphism did not differ between cases and controls. The frequency of the x allele of the XbaI polymorphism among breast cancer patients, however, was 1.4 times of that for controls (95% CI, 1.0–1.9; Ref. 12). Among the breast cancer patients, there was an association of borderline significance between the XbaI restriction site and older age at onset. In a hospital-based case-control study (201 cases and 201 controls) conducted in South Korea, Shin et al. [19] reported that OR associated with the xx genotypes of XbaI was 2.38 (95% CI, 1.58–3.58) compared with women with XX genotype. Additionally, Ladd et al. [20] performed meta-analyses of published data to examine the effect of both polymorphisms. These meta-analyses also suggest there are no differences in risk among genotype groups of these two ER α variants and that the ER α polymorphisms do not play a role in breast cancer risk in Caucasian postmenopausal women.

Menarche is regulated by a variety of environmental and genetic factors. Twin analyses have estimated that genetic effects may be more important parameters [41],[42]. Such studies have indicated that 53-74% of the variation in age of menarche may be attributed to genetic effects. Estrogen exposure of tissues mediated via the estrogen receptor (ER) may be an important determinant of menarche and may be genetically determined in this regard. Conversely, the age of menarche may then influence the total duration of tissue estrogen exposure.

In this study, it was found that there was statistically significant decrease in the age of menarche of individuals with presence of PvuII restriction site (Pp and pp genotypes) compared with individuals with absence of restriction site (PP genotype) ($P = 0.021$). Also, there was statistically significant decrease in the age of menarche of individuals with presence of XbaI restriction site (Xx and xx genotypes) compared with individuals with absence of restriction site (XX genotype) ($p < 0.001$). These results are consistent with that of Karapanou, and Papadimitriou, who reported a delay in the age of menarche in subjects homozygous for PX haplotypes [43]. Also XX homozygotes are protected from breast cancer and endometrial cancer [18],[44]. The biological pathway for XbaI and PvuII that may affect the age of menarche is not well known. However, the PX haplotype may be important in regulating not only the onset, but also the end of high tissue estrogen exposure during the lifetime of an individual.

So, the researchers concluded the existence of common genetic factors as well as disease susceptibility differences for

breast cancer in populations and highlighted the importance of performing comparison analyses for disease susceptibility within ethnic populations [4],[45],[46].

VI. CONCLUSION

The combined analysis of multiple parameters in this study supported the presence of genetic polymorphisms that are associated with the risk of breast cancer. This may help to assess the risk individual more precisely for early detection of breast cancer and to select the candidates for hormonal and chemoprevention more efficiently. Further large scale studies are required to verify the results of our study.

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