

Reduction in breast cancer susceptibility due to *XbaI* gene polymorphism of alpha estrogen receptor gene in Jordanians

Manar Fayiz Atoum
Foad Alzoughool

Department of Medical Laboratory
Sciences, Faculty of Allied Health,
Hashemite University, Zarqa, Jordan

Abstract: Breast cancer is a global health concern among women worldwide. Estrogen receptor alpha (ER α) mediates diverse polymorphic effects in breast tissues that may relate to breast cancer susceptibility. The aim of this study was to evaluate the effect of -397 *PvuII* (T/C) and -351 *XbaI* (A/G) restriction fragment length polymorphism within intron 1 of ER α , and its effect on breast cancer susceptibility. A total of 156 women who were histopathologically diagnosed with breast cancer and 142 healthy Jordanian women were enrolled in this case-control study. Genomic DNA was extracted from whole peripheral blood, and the desired fragment was amplified using polymerase chain reaction followed by restriction digestion with *PvuII* and *XbaI* restriction enzymes. The results showed no significant association between *PvuII* polymorphism and breast cancer risk. However, a significant association was found between *XbaI* polymorphism and reduction in breast cancer risk within the “x” allele of heterozygotes (odds ratio [OR] 0.199, 95% confidence interval [CI] 0.09–0.044) and heterozygotes (OR 0.208, 95% CI 0.09–0.047). The combined analysis of *PvuII* and *XbaI* polymorphisms revealed a synergistic effect of Pp/Xx and pp/xx genotypes and a significant reduction in breast cancer risk with these genotypes. The results also showed no statistical differences among *PvuII* or *XbaI* polymorphisms based on stage, ER, progesterone receptor and expression of hormone receptor such as human epidermal growth factor receptor 2. This case-control study showed that *XbaI* polymorphism of alpha estrogen gene modified and reduced breast cancer susceptibility among Jordanians.

Keywords: breast cancer, gene polymorphism, *XbaI*

Introduction

Breast cancer is a serious life-threatening condition affecting women worldwide.¹ It ranks second among various causes of cancer death in women.² In Jordan, 42.9% of cancers diagnosed in women are breast cancers.³ Understanding the pathogenesis of breast cancer is important in the innovative therapies for breast cancer diagnosis and treatment. One of the most important risks of breast cancer is endogenous hormone level. The genes of estrogen receptor (ER) pathway may influence breast cancer risk by two possible mechanisms: 1) ER-mediated stimulation of breast cell proliferation with a concomitant enhanced rate of mutations and 2) metabolism of estradiol to genotoxic metabolites with a resulting increase in DNA mutations.⁴

Estrogen is a steroid hormone that is essential for the development of female secondary sexual characters.⁴ Under normal conditions, it affects the growth, differentiation and function of breast,⁵ uterus, vagina, ovary, testis, epididymis and prostate.⁶ However, under abnormal conditions, exposure to estrogen predisposes females to a risk of developing breast cancer. Current hormonal therapies have benefited breast

Correspondence: Manar Fayiz Atoum
Department of Medical Laboratory
Sciences, Faculty of Allied Health,
Hashemite University, Zarqa 13115, Jordan
Tel +962 79 973 3894
Email manar@hu.edu.jo

cancer patients. However, their success is often limited to patients whose tumors express ER α , but entirely ineffective in ER-negative breast cancers.⁷

ERs are of special interest as their expression is always affected in malignant cancer cells.⁸ ERs are nuclear receptor proteins that have an estrogen-binding domain and a DNA-binding domain.⁹ There are two types of ERs: 1) ER α that is localized on 6q25.1 and associated with breast cancer and 2) ER β that is located on chromosome 14q22-24 and functions as a tumor suppressor.¹⁰ Polymorphisms in the ER genes may determine the biosynthetic activity of circulating estrogen.

Nearly over half of all types of breast cancers overexpress ER α and ~70% of these respond to anti-estrogen.¹¹ Elevated levels of ER α in benign breast epithelium indicate an increased risk of breast cancer.¹² ER α status is essential in making decisions about endocrine therapy with anti-estrogen since ER α (+) status correlates with improved prognosis and better overall survival.⁹

ER α is regarded as one of the significant prognostic factors for breast, ovarian and lung cancers,¹³ miscarriage and endometriosis. Single nucleotide polymorphisms were associated with increased risk of cancers.¹⁴ The most characterized single nucleotide polymorphisms of ER α located in the first intron are the c454-397T>C- and c454-351A>G-site polymorphisms. These polymorphisms are 397 and 351 base pairs upstream of exon 2 and are detected by restriction enzymes *PvuII* or *XbaI*, respectively.¹⁵ These polymorphisms were correlated with breast cancer incidence.¹⁶ *PvuII* altered protein expression by interfering with ER α mRNA splicing, but the mechanism of action of *XbaI* is still unknown.

Genetic studies on cancer are scarce in Jordan and only few studies have screened gene polymorphism in breast cancer.¹⁷⁻²⁰ To our knowledge, no studies have determined the role of ER α *PvuII* and *XbaI* polymorphisms in breast cancer susceptibility among Jordanians. Therefore, the aim of this study was to determine the frequency of ER α *PvuII* and *XbaI* genotypes and the possible involvement of these polymorphisms in breast cancer susceptibility among Jordanian women with breast cancer.

Materials and methods

This study enrolled 156 women who were histologically and clinically diagnosed with breast cancer and 142 age-matched controls with no previous family history of any cancer from Al-Basheer Hospital (2013–2015). International review board approval was obtained from the Hashemite University international review board committee and signed consent

forms were obtained from all patients. All clinical samples were staged according to Atoum et al.²¹

Genomic DNA was extracted from all blood samples using Wizard extraction kit (Promega, Madison, WI, USA) and was stored at -80°C . Polymerase chain reaction was carried out to amplify intron 1–exon 2 region of ER α gene in three steps with annealing at 60°C in a thermal cycler (Bio-Rad, Munich, Germany) in 25 μL reaction volume. Each reaction mixture contained 10 pmol of each primer, 3 mM of MgCl_2 , 100 nM of each deoxynucleotide triphosphate, 1 unit of Taq DNA polymerase and 100 ng of genomic DNA. Primer sequences are as follows: forward primer: 5' AGG GTT ATG TGG CAA TGA CG 3'; reverse primer: 5' CCT GCA CCA GAA TAT GTT ACC T 3'. The amplified fragment of 1374 base pairs was then digested with *PvuII* and *XbaI*. The presence of *PvuII* restriction site on both alleles (pp) was identified by two fragments (850, 450), while the presence of *XbaI* on both alleles (xx) was identified by two fragments (900, 400). Digested fragments were then electrophoresed on 1.5% agarose gel with ethidium bromide.

Statistical analysis

Statistical analysis was carried out using SPSS software. The distribution of genotypes in cancer patients and controls was evaluated by the Chi-square test. Odds ratio (OR) and 95% confidence interval (CI) were used to estimate the relative risk. A p -value <0.05 was considered statistically significant.

Results

Genotypic distribution of ER α *PvuII* and *XbaI* is shown in Table 1. With reference to PP genotype, no significant associations were found between the variations of breast cancer risk and P alleles in homozygotes or heterozygotes. There was no statistical difference in the prevalence of P allele between

Table 1 Frequency of estrogen receptor alpha polymorphism *PvuII* and *XbaI* among breast cancer patients and controls

Genotype	Cases n=156, n (%)	Controls n=142, n (%)	OR	95% CI	p-value
PP	24 (15.4)	19 (13.4)	1	NA	NA
Pp/pp	132 (84.6)	123 (86.6)	1.18	0.61–2.25	0.62
Pp	87 (55.8)	82 (57.7)	1.19	0.61–2.33	0.61
pp	45 (28.8)	41 (28.9)	1.15	0.55–2.40	0.71
XX	39 (25)	9 (6.3)	1	NA	NA
Xx/xx	117 (75)	133 (93.7)	0.203	0.09–0.43	0.0001
Xx	71 (45.5)	82 (57.7)	0.199	0.09–0.44	0.0001
xx	46 (29.5)	51 (35.9)	0.208	0.09–0.47	0.0002

Note: Significant p -value <0.05 .

Abbreviations: CI, confidence interval; OR, odds ratio; NA, not available.

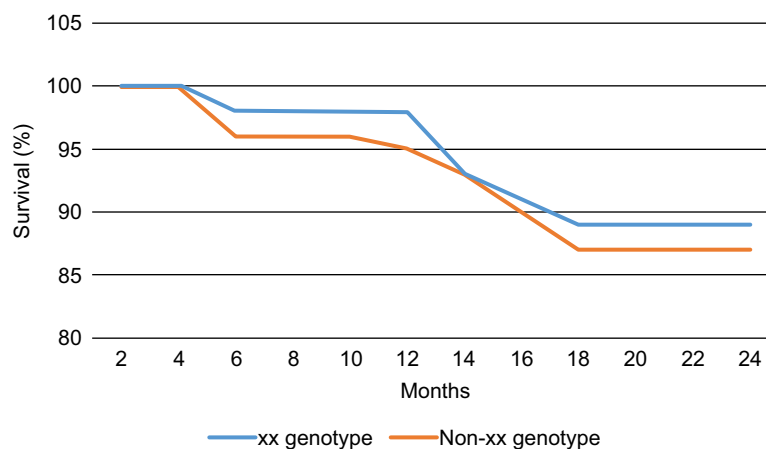


Figure 1 Kaplan–Meier survival plot showing differences in survival probability among xx breast cancer genotype compared to non-xx genotype.

cases (84.6%) and controls (86.6%). Nearly 15.4% of the patients and 13.4% of the controls were homozygous for P allele. About 55.8% of the patients and 57.7% of the controls were heterozygous for this allele. No significant association was found between breast cancer risk and P allele in heterozygotes (OR 1.19, 95% CI 0.61–2.33) or homozygotes (OR 1.15, 95% CI 0.55–2.40). A statistical difference was observed in the prevalence of X allele between cases (75%) and controls (93.7%). *XbaI* polymorphism in x allele was more prevalent among controls (35.9%) compared to patients with breast cancer (29.5%), (OR 0.208, 95% CI 0.09–0.47).

Combined analysis of *PvuII* and *XbaI* genotypes revealed a synergistic effect of Pp/Xx (OR 0.11, 95% CI 1.19–9.66, $p = 0.03$) and pp/xx (OR 0.11, 95% CI 0.02–0.58, $p = 0.01$) as carriers of these genotypes showed reduced risk of breast cancer (Table 2).

Table 3 shows the histological subtypes of different *PvuII* and *XbaI* genotypes among women with breast cancer. No statistical difference was found within either *PvuII* or *XbaI* genotypes ($p = 0.83$, 0.81, respectively) among different breast cancer stages.

Table 2 Breast cancer risk in relation to *PvuII* and *XbaI* combination genotypes

Genotype	Cases	Control	OR	95% CI	p-value
PP/XX	8	2	1	NA	NA
PP/Xx	10	8	0.31	0.05–1.90	0.20
PP/xx	6	14	0.107	0.01–0.66	0.16
Pp/XX	23	4	0.70	1.19–29.66	0.70
Pp/Xx	35	52	0.11	1.19–9.66	0.03
Pp/xx	29	31	0.28	0.04–1.19	0.08
pp/XX	8	3	0.67	0.08–5.12	0.69
pp/Xx	26	2	3.25	0.39–26.91	0.27
pp/xx	11	26	0.11	0.02–0.58	0.01

Note: Significant p-value <0.05.

Abbreviations: CI, confidence interval; OR, odds ratio; NA, not available.

Table 3 Breast cancer patient stages among *PvuII* and *XbaI* genotypes

Cancer genotype	Stage I	Stage II	Stage III	Stage IV	Total	p-value
PP	1	7	11	5	24	0.83
Pp	1	22	35	29	87	
pp	1	12	21	11	45	
XX	0	11	19	9	39	0.81
Xx	2	20	37	12	71	
xx	0	14	23	9	46	

Note: Significant p-value <0.05.

Table 4 Estrogen receptor, progesterone receptor and hormone receptor HER2 among breast cancer cases

Cancer genotype	ER positive	PR positive	HER2 positive	p-value
PP	13	17	9	0.19
Pp	48	51	11	
pp	21	28	13	
Total	82	96	33	
XX	11	15	7	0.43
Xx	53	50	12	
xx	23	26	10	
Total	87	91	29	

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

No statistical differences were found among *PvuII* or *XbaI* polymorphisms based on ER, progesterone receptor and expression of hormone receptor such as human epidermal growth factor receptor 2 (HER2) (Table 4).

Discussion

This case–control study was conducted to analyze the association between *PvuII* and *XbaI* polymorphisms and breast cancer risk among Jordanian females. No association between the variation of breast cancer risk and pp genotype was found

compared to PP genotype, but a significant association was found between reduced breast cancer risk and xx genotype compared to XX genotype. Also no association was found between the variation of breast cancer risk and *PvuII* or *XbaI* genotypes based on breast cancer stages, presence of ER, progesterone receptor and expression of hormone receptor HER2 among breast cancer females. The results of this study are in contrast with those of a Chinese study that analyzed *PvuII* and *XbaI* for breast cancer risk. A significant association was found between Pp and pp genotypes and increased breast cancer risk, whereas no significant association was found between Xx and xx genotypes and increased breast cancer risk.²² The results of this study are also in contrast with the results of another Chinese study that was conducted among another Chinese group²³; the results showed no effect of *PvuII* and *XbaI* polymorphisms on breast cancer risk. This study showed a significant association between reduced breast cancer risk and xx genotype, in contrast to the results of a study on Pakistani breast cancer patients,²⁴ and similar to the results of a study on Portuguese population where xx genotype was associated with decreased breast cancer risk.¹⁶ This contradiction may be due to the large and different biological processes where alpha estrogens and their receptors are involved. The possible mechanisms by which *PvuII* and *XbaI* polymorphisms affect breast cancer are still not known. The position of *PvuII* and *XbaI* polymorphisms in the intron near the promoter may contain regulatory sequences like enhancers that affect the expression of alpha receptor gene. Intronic enhancers act as regulatory sequences that control transcriptional regulation.²⁵ Nowadays, ER α has been signaled as a possible marker for many diseases, including different cancers and immunological diseases.

Conclusion

The results of this case–control study showed a significant reduction in breast cancer risk in women carrying xx genotype. So further studies are needed with larger sample size among different populations to clarify how ER α and *XbaI* may modify an individual's susceptibility to breast cancer risk.

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Disclosure

The authors report no conflicts of interest in this work.

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