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Association between Estrogen Receptor alpha Polymorphisms and Breast Cancer Risk in Jordanian Women

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Abstract

Estrogen receptor alpha (ER-α) belong to the nuclear receptor superfamily of ligand-inducible transcription factors. It is involve in both mammary development and in breast carcinogenesis. Many polymorphisms suggested association with the incidence of breast cancer. The most widely studied Polymorphisms are PvuII and XbaI restriction sites, they lies within intron one of estrogen receptor alpha gene. This study was done to evaluate the relationship of two well- studied polymorphisms in the estrogen receptor alpha gene, (PvuII and XbaI), and the risk of breast cancer in Jordanian women. In our study, 100 blood samples from Jordanian women with breast cancer had been obtained and used for subsequent DNA extraction and genotyped for the PvuII and XbaI polymorphisms using Restriction Fragment Length Polymorphism (RFLPs) technique. Also, a 100 blood samples from normal Jordanian women had been used as control. For statistical analysis, SPSS computerized program to calculate P-value for polymorphisms and Hardy-Weinberg equation for genotype frequencies was used. There was no significant difference between breast cancer patients and healthy control individuals in the distribution of the PvuII genotypes. There was no significant difference between breast cancer patients and healthy control individuals in the distribution of the PvuII genotypes ($P>0.05;P=0.27$), no association between XbaI polymorphism and breast cancer risk ($P>0.05;P=0.111$). Also there was no significant association with haplotype analysis ($P>0.05;P=0.07$) except for AGTC haplotype ($P\leq0.05;P=0.043$) and AATC haplotype ($P\leq0.05;P=0.027$). This result suggests that PvuII and XbaI polymorphism doesn't play a role in the susceptibility to breast cancer in Jordanian women.

Keywords: Estrogen receptor, polymorphism, Breast Cancer

INTRODUCTION

Breast cancer is the most common cancer among women and the second leading cause of cancer death in women after lung cancer. And one from every 8 -9 women well develops the disease in the western and northern Europe. It is currently accounted for 20% of all female cancers worldwide [1, 2].

Ethnicity also plays role in the risk of breast cancer, with the incidence varying from lowest in certain groups of Asian women to highest in Caucasian women. Breast cancer also called ductal carcinoma because mammary carcinogenesis begins with the proliferation of epithelial cells called ductal cells.

There are risk factors cannot be changed, include age and gender (the majority of cases in women over age 50) , family history of breast cancer (about 20 – 30 % of patients have family history of the disease) , genes (the most common gene defect are found in BRCA 1 and BRCA 2 genes) and menstrual cycle (women who get their period early " before age 12 " or went through menopause late " after age 55 " have on increased risk for the disease [3,4] . According to the American cancer society, about 1.3 million women diagnosed with breast cancer worldwide, about 465,000 will die from the disease. In Jordan Breast cancer is the most common cancer overall as well as the most common malignancy afflicting women, according to the latest statistics from the Jordan National Cancer Registry, 674 females and 10 males were diagnosed with breast cancer in 2005, accounting for 18.6% of the total new cancer cases . Breast cancer ranked first among cancer in females, accounting for 36.2% of all female cancers, and is the leading cause of cancer deaths among Jordanian women. (announced by the Jordanian ministry of health).

The risk effect of estrogen on breast cancer was first shown when researchers found that removing the ovaries of women with breast cancer improving the chance of survival [5]. Recently many researchers have examined the possible relationship between exposure to estrogen and breast cancer [6, 7, 5, 8- 9]. One recent study showed that women who developed breast cancer tended to have higher levels of estrogen circulating in their bodies than women without breast cancer [10]. Another recent study showed that women who had been treated for breast cancer, and who had higher levels of estrogen in their bodies, had a return of the disease sooner than women treated for breast cancer and who had lower levels of estrogen [10, 11].

This evidence suggests that long exposure to estrogen during the life plays an important role in determining breast cancer risk [6, 7, 5, 8, 9, 10, 12- 11]. Studies that have identified risk factors for breast cancer have found that women who experience menarche at an early age, or menopause at a later have a higher risk of breast cancer [13, 14, 15].This also supports the theory that the number of menstrual cycles a woman has and the length of exposure to estrogen during her lifetime affects her risk for breast cancer [13, 14, 15]. Physiologically, the female breast is under control of many hormones; estrogen is responsible for the development of the ductal system, whereas progesterone is necessary for lobular development, invasive duct carcinoma (IDC) is the commonest form of breast cancer accounting for 85-90% of all cases, so this signify the important role of estrogen in breast cancer [7]. Estrogen is involved in both normal mammary development and in breast carcinogenesis [16, 17, 18- 19]. Epidemiological studies have shown that exposure to estrogen is a risk factor for breast cancer [16, 17, 18, 19, 20]. Estrogen exerts its biological effects though the estrogen receptor alpha (ER-α) and the more recently discovered estrogen receptor beta (ER-β) [20, 21- 22]. ER-α and ER-β belong to the nuclear receptor superfamily of ligand-inducible transcription factors.

Estrogen receptors possess high sequence similarity in their DNA- and ligand – binding domains, but diverge at the transcriptional activation domain, means that they target different genes [23]. also ER-α has higher affinity for estrogen [23] .only a small fraction (<5%) of women with breast cancer have a clear hereditary predisposition , and of these due to mutation in BRCA1 and BRCA2 genes. so there are other genes (factors) act as modifier of breast cancer. one of these genes is estrogen receptor gene , which locates on chromosome 6 (6q 25.1) [18,19,20,21-22] .These receptors contain a DNA – binding domain , dimerization domain, hormone-binding domain and transcription activation domain [23,21]. estrogen diffuse passively through cell and nuclear membranes and binds to it's receptor structure, that permits the subsequent dimerization and interaction with coactivator molecules , which trigger gene activation .This mechanism activate gene transcription and synthesis of specific proteins that can influence cell growth in breast epithelial tissues [16,17,18-23] . The ER-α gene spans 140 Kb and is comprised of 8 exons , which are spliced to yield 6.3 Kb mRNA, has length of 1783nts encoding a595 amino acids protein with a molecular weight of 66 Kd , comprised the different domains [20]. Where the transcription activation domain lies within exon 1 and exon8, DNA binding domain of~70 amino acids is located in exon2 and exon3. Exon4 through 8 translating into 250 amino acids in size are necessary and sufficient for ligand binding, 22 amino acid sequence necessary for subunit dimerization has been located within exon7 [20,21].Transcription of ER-α gene occurs from at least two different promoters, with the distal promoter (promoter B) located 2 Kb upstream of the proximal one (promoter A). The resultant transcripts from the two promoters differ only in the non-coding region at the 5'-end, and both types of ER-α mRNA encodes the same protein [20, 21].

Many epidemiological studies have shown that prolonged or increased exposure to estrogen increases the risk of breast cancer [16, 17, 18, 19- 20]. A common phenotype in breast cancer is the expansion of ER-α cell population and inappropriate elevation of ER-α protein [25].

MATERIALS AND METHODS

The Breast Cancer Patients Samples

Peripheral blood samples were collected from one hundred female patients histopathologically diagnosed for breast cancer during 2009-2010. The mean age for patients is 52.3, with age ranged from 30 to 75 years.

The breast cancer samples with the necessary information were collected from the clinic of chemotherapy before chemotherapy drugs have been administrated to the patients at King Abdullah University Hospital, Prince Basmah Hospital in Irbid, and Al-Basheer Hospital in Amman. Complete medical histories were collected from the medical records.

The Controls Samples

For genotypes frequencies and distribution purposes in the normal population, one hundred female blood samples were collected from healthy Jordanian female at King Abdullah University Hospital in Irbid. The individuals in the control group had various diagnoses in the hospital excluding cancer and had no cancer history. The mean age was 50.5, and ranged from 30 to 95 years. (Table .1)

Genomic DNA Extractions

Genomic DNA of all participants was isolated from whole blood using Wizard Genomic DNA Purification kit according to the manufacturer's recommendations. (Promega, USA)

Amplification of Samples DNA Using PCR

Detection of the polymorphic allelic variants, genotyping ER-α gene was performed using thermal cycler (BioRad, Italy) and reagents from (Promega, USA). Oligonucleotide primers (Fermentas, USA) were used for amplification purposes. (Table. 2)

Genotyping of ER-α Gene

Genotyping ER-α gene PvuII and XbaI genotypes was performed by PCR based restriction fragment length polymorphism (PCR-RFLP). The presence of PvuII SNP (C to T) in the intron one of ER-α and the presence of XbaI SNP (G to A) 45bp away from PvuII in the same intron was investigated by PCR-RFLP.

Table 1. Selected characteristics frequencies for breast cancer patients and controls.

Characteristics	Patients	Controls
Age	30-39: 15 40-49: 25 50-59: 23 60-69: 28 70-79: 09	30-39: 16 40-49: 23 50-59: 33 60-69: 20 70-79: 08
Occupation	Workers: 23 Housewives: 77	Workers: 19 Housewives:81
Smokers	Yes: 17 No: 83	Yes: 11 No: 89
Sex	Female: 100 Male: 0	Female: 100 Male: 0
History	All cancers: 36 Breast cancer: 11	No data

Table 2. PCR oligonucleotide primers of the intron one of the ER-α Gene.

Sequence of primers used for estrogen receptor alpha genotyping				
SNP site	Primer site	Annealing temperature	Restriction enzyme	Allele size
Intron1 T/C	5'-ctgccaccctatctgtatcttttcctattctcc-3' 5'-tctttctctgccaccctggcgtcgattatctga-3'	64	PvuII	T: 936+438 C: 1374
Intron 1 A/G	5'-ctgccaccctatctgtatcttttcctattctcc-3' 5'-tctttctctgccaccctggcgtcgattatctga-3'	64	XbaI	A: 981+393 G: 1374

The PCR reaction mix consist of 12.5 µl PCR master mix (Promega, USA) containing; {5 units/µl Taq DNA polymerase, supplied in a propriety reaction buffer, PH 8.5},{400 µM of each dNTP;dATP,dCTP,dGTP,dTTP} and {3 mM MgCl2}. Reaction mix also contains 1.3µl of forward and 1. 3µl of backward primers (1 pmole/µl). 3µl of genomic DNA. 7.9µl nuclease free water for a reaction volume of 25µl.

The PCR temperature profile consist of 1 cycle of 94°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, 62°C for 1 minute, 72°C for 1 minute. Ending with an elongation step at 72°C for 5 minutes. PCR products (1374bp) were then subjected to restriction enzyme (endonuclease enzyme that recognize specific palindromic DNA sequence and cleave the double strands at specific site called recognition site, leading to different fragments with different sizes). Digestion with PvuII (Fermentas, USA) and XbaI (Fermentas, USA) at 37°C for 4 hours, as follows: 0.3µl (10 units/µl) PvuII or XbaI restriction enzyme, 7.7µl nuclease free water, 2µl {10X} restriction enzyme buffer (G buffer for PvuII and Tango buffer for XbaI), and 10µl PCR product for 20µl reaction volume.

We have used positive controls for the digestion reactions consisting of a DNA from two mice genomes (from Hadasa hospital-West bank), for the XbaI restriction enzyme the amplified product is 1141 bp, after digestion, produce 955 bp and 186 bp. The PvuII positive control produce 1142 bp after amblification with PCR, when digested with PvuII give 444bp and 698 bp. (Figure 1.1) &(Figure 1.2)

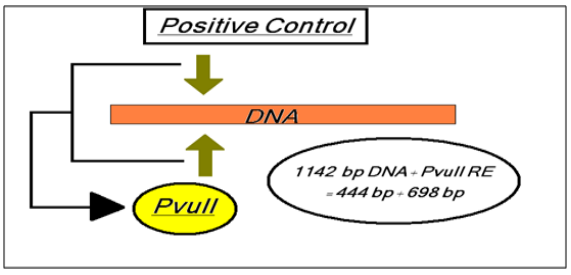

Fig.1.1. Restriction map for the positive control for PvuII restriction enzyme.

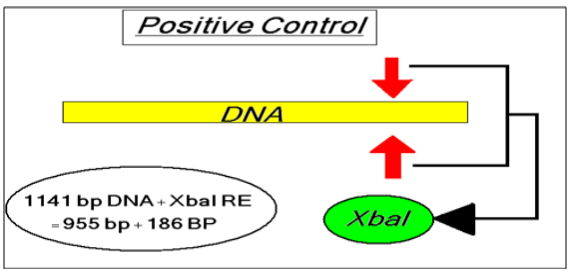

Fig.1.2. Restriction map for the positive control for XbaI restriction enzyme.

Agarose Gel Electrophoresis

Genomic DNA, PCR amplification products and restriction products were detected by performing agarose gel electrophoresis. For detection of the genomic DNA extracted from whole blood, 4µl of genomic DNA were mixed with 2µl 6X Orange loading dye.

Loaded in 1% agarose gel and run side by side with 0.05µg/µl Orange Ruler marker (Fermentas, USA) in 0.5X TBE buffer at 100 Volt for less than hour. ER-α intron one PCR product was detected by 2% agarose gel run in 0.5X TBE buffer at 100 Volt for 1.5 hour. PvuII and XbaI digested PCR products were loaded in 3% agarose gel and run in 0.5X TBE buffer at 100 Volt for 3 hours. All gels were stained with (10mg/ml) ethidium bromide (Biorad, USA) and visualized by using UV transilluminator (Biorad, USA) The fragment size of each PCR and digested products were determined by comparison with 7µl 200bp DNA molecular weight marker (Fermentas, USA).

Statistical Analysis

To assess the significance of the association of ER-α gene polymorphisms and the breast cancer we used a statistical analysis method using Chi-square to calculate P-value, which is a biomedical indicator for importance of the correlation between two factors (patients and controls). Statistically, if the calculated P value ≤ 0.05, this means that there is a significant difference between breast cancer patients and controls. If P-value > 0.05, this means that there is no difference between breast cancer patients and controls.

RESULTS

Detection of the intron 1 of ER-α gene PvuII and XbaI polymorphisms using RFLP technique

DNA was extracted from the blood samples. Then PCR amplification used to produce quantitative amounts of intron 1 of ER-α gene with 1374 bp size. (Figure 2).

Due to its simplicity, rapid, inexpensive and quantitatively detected procedure, RFLPs method was used to investigate the presence of our research polymorphisms PvuII and XbaI., these two polymorphisms are 46 bp apart and exhibit strong linkage disequilibrium (LD). For genotyping of these polymorphisms in our samples, two reaction tubes were used; the first tube for PvuII genotyping and the second for XbaI genotyping. (Table .3)

PvuII recognize hexamer recognition site (5'… CAG▼CTG…3'), if T allele is present, the 1374 bp PCR product is sensitive for digestion and produce 936 bp and 438 bp bands, if C allele is present, the 1374 bp PCR product well resist the digestion and produce intact 1374 bp band .(Figure 3).

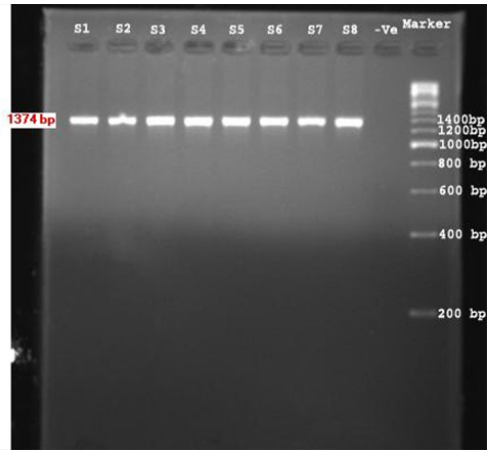

Fig 2. Electrophoretogram represent 2% agarose gel electrophoresis for Estrogen Receptor Alpha (ER-α) PCR product (1374 bp) from breast cancer patients samples. Lanes S1 to S8 represents PCR products from breast cancer patients.

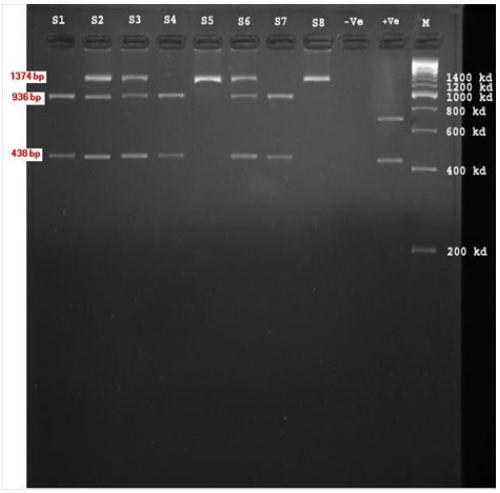

Fig 3. Electrophoretogram representative 3% agarose gel electrophoresis for Estrogen Receptor Alpha (ER-α) PCR product (1374 bp) from breast cancer patients digested with PvuII restriction enzyme.

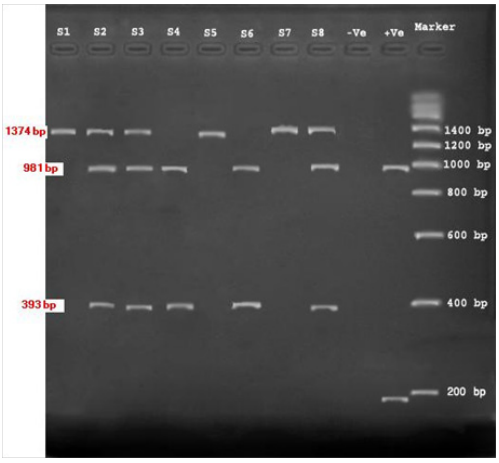

Fig 4. Electrophoretogram representative 3% agarose gel electrophoresis for Estrogen Receptor Alpha (ER-α) PCR product (1374 bp) from breast cancer patients digested with XbaI restriction enzyme.

In XbaI genotyping, the 1374 PCR products from 200 samples were digested with XbaI restriction enzyme. After 4 hours incubation at 37°C, all digested products from all samples were loaded in 3% agarose gel electrophoresis for 3 hours. (Figure 4).XbaI recognize hexamer recognition site (5'…T▼CTAGA…3'), if A allele is present, the 1374 bp PCR product is sensitive for digestion and produce 981 bp and 393 bp bands, if G allele is present, the 1374 bp PCR product well resist the digestion and produce intact 1374 bp band.

When the results of all samples after digestion with PvuII and XbaI restriction enzymes have been documented, SPSS computerized program (version 16.0) was used to assess the significance of ER-α gene polymorphisms and the breast cancer. This method using Chi-square to calculate P-value. When genotype analysis have been conducted, PvuII SNP was equally distributed between patients and controls with P-value = 0.27 (P-value > 0.05), which means no significant association between the presence and absence of the SNP (Table. 4 A).

Table 3. Description of the restriction enzymes and the lengths of the polymorphic DNA fragments of the intron one of ER-α gene.

Gene	Size of PCR products	Restriction enzyme	Restriction sequence	Size of polymorphic DNA sequence
ER-α (intron one)	1374 bp	PvuII	5'…CAG▼CTG…3' 3'…GTC▲GAC…5'	1374 bp no cut 936+438 bp cut
ER-α (intron one)	1374 bp	XbaI	5'…T▼CTAGA…3' 3'…AGATC▲T…5'	1374 bp no cut 981+393 bp cut

Table.4A. The distribution of PvuII genotypes among breast cancer Patients and the control group.

PvuII genotypes	Patients		Controls	
	Expected	Observed	Expected	Observed
TT	32.49	29	26.52	27
TC	49.02	56	49.96	49
CC	18.49	15	23.52	24
P-value	0.27 (no significant)			

For XbaI SNP, statistics relieved that there was no significant association between the presence and absence of the SNP with P-value = 0.111 (P-value > 0.05) (Table. 4 B).

The genotype distributions for PvuII SNP was assessed to be in Hardy-Weinberg equilibrium for both patients and controls. But the XbaI SNP was out of Hardy-Weinberg equilibrium expectation for patient samples .Haplotype analysis on nine haplotypes also have been conducted for association with breast cancer. A-G-T-C haplotype was shown to have a significant increase in breast cancer risk (P-value = 0.043; P-value ≤ 0.05). A-A-T-C haplotype have a significant protection against breast cancer (P-value = 0.027; P-value ≤ 0.05). The other 7 haplotypes have no association with breast cancer (P-value > 0.05) (Table. 5).

The frequency of PvuII and XbaI alleles between patients and controls were equally distributed. For PvuII (P-value = 0.478; P-value > 0.05). For XbaI (P-value = 0.887; P-value > 0.05 (Table 6).

Among 100 breast cancer patients recruited for our study, 36 patients were with a history of cancer in their families. PvuII and XbaI genotypes distribution was tested between those patients and controls, no significant association was shown. For PvuII (P-value = 0.107; P-value > 0.05). For XbaI (P-value = 0.116; P-value > 0.05) (Table 7).

The distribution of PvuII and XbaI genotypes between the other 64 breast cancer patients (without history of cancer) and controls was tested, no significant association was shown. For PvuII (P-value =0.310; P-value > 0.05). For XbaI (P-value = 0.158; P-value > 0.05) (Table 8).

Table 4B. The distribution of XbaI genotypes among breast cancer Patients and the control group.

	XbaI genotypes	Patients		Controls
	Expected	Observed	Expected	Observed
AA	29.16	20	28.09	26
AG	49.68	68	49.82	54
GG	21.16	12	22.09	20
	P-value	0.111 (no significant)		

Table 5. Distribution of combined PvuII and XbaI genotypes (haplotypes) among breast cancer patients and control group.

Haplotype	Patients	Controls	P-value
AG,TC	47 (47%)	33 (33%)	0.043
AA,TT	17 (17%)	13 (13%)	0.428
AG,TT	12 (12%)	13 (13%)	0.831
AA,TC	3 (3%)	11 (11%)	0.027
AG,CC	9 (9%)	8 (8%)	0.800
GG,CC	6 (6%)	14 (14%)	0.059
GG,TC	4 (4%)	5 (5%)	0.733
GG,TT	2 (2%)	1 (1%)	0.561
AA,CC	0 (0%)	2 (2%)	0.155
P-Value			0.07

Table 6. Distribution of PvuII and XbaI alleles among breast cancerPatients and controls.

	Allele frequency			
	PvuII		XbaI	
	T	C	A	G
Patients	0.57	0.43	0.54	0.46
Control	0.52	0.48	0.53	0.47
P-value	0.478		0.887	
Population	0.54	0.46	0.54	0.46

Table 7. The distribution of PvuII and XbaI genotypes among 36 breast cancer patients with a history of cancer in their families and controls.

PvuII SNP	Patients	Controls	P-value	XbaI SNP	Patients	Controls	P-value
TT	6	27	0.107	AA	4	26	0.116
TC	25	49		AG	26	54	
CC	5	24		GG	6	20	
Total	36	100		Total	36	100	

Table 8. The distribution of PvuII and XbaI genotypes among 64 breast cancer patients (without cancer history in their families) and controls.

PvuII SNP	Patients	Controls	P-value	XbaI SNP	Patients	Controls	P-value
TT	23	27	0.310	AA	16	26	0.158
TC	31	49		AG	42	54	
CC	10	24		GG	6	20	
Total	64	100		Total	64	100	

DISCUSSION AND CONCLUDING

Long exposure to estrogen categorized as a risk factor for breast cancer through the binding of estrogen to estrogen receptor in the mammary tissue, which lead to proliferation and differentiation. Also many epidemiological studies have shown that prolonged or increased exposure to estrogen increases the risk of breast cancer [24].

The effects of estrogen are mediated through the intracellular estrogen receptors; nuclear receptor proteins, that act as ligand-inducible transcription factor with DNA-binding domain, dimerization domain, hormone-binding domain and several transcriptional activation domains. The estrogen-estrogen receptor interaction leads to stimulation of cell growth in breast epithelial tissues [25].

As many previous studies in different countries, two genetic polymorphisms in the ER-α have been studied in Jordan University of Science and Technology (JUST); Rs2234693 (PvuII) and Rs9340799 (XbaI) polymorphisms, for there relation with breast cancer [24].

The two most frequently studied variants are the single nucleotide polymorphisms (SNPs) rs2234693 (known as PvuII T→C) and rs9340799 (known as XbaI A→G). Both SNPs are located in the intron one of the estrogen receptor alpha gene. Different studies reported statistically significant association between ER-α polymorphisms and breast cancer [26, 18, 24, 27-28], while other studies including our study haven't [29, 30].

A number of studies have been published, the Korean hospital-based study with 205 confirmed breast cancer cases and 205 age-matched controls, which reported a significant decrease of breast cancer risk with XbaI SNP G allele (AG,GG) when compared with women without G allele (AA) [24,28]. The same Korean study didn't find any association between breast cancer and PvuII SNP [24, 28].

Similarly, the Norway hospital-based study with 360 breast cancer patients and 672 controls reported an increase in breast cancer risk associated with A allele of XbaI polymorphism and the G allele as protective allele [26, 24]. The same Norway study didn't find any association between breast cancer and PvuII SNP [26, 24].

In the Shanghai breast cancer study with 1069 breast cancer patients and 1169 controls, they reported the A allele of XbaI polymorphism to be associated with a nonsignificantly elevated risk in postmenopausal women [26, 24]. The same study reported that genotypes with T allele (TT,TC) significantly associated with breast cancer when compared with CC genotype [26,24].

In a Swedish breast cancer case-control study with 1556 breast cancer patients and 1512 controls, they did not find any association between breast cancer and XbaI polymorphism [24, 29]. Also they did not find any association between breast cancer and PvuII polymorphism [24, 29].

In a Caucasian study with 393 breast cancer patients and 790 contros, they reported a significant association between breast cancer and genotypes with A allele (AA,AG) when compared with GG allele [24]. The same study reported no association between braest cancer and PvuII polymorphism [24].

In a study between US white and African-American with 220 breast cancer patients and 192 controls, they didn't find any association between braest cancer and XbaI polymorphism [31]. The same study didn't find any association between breast cancer and PvuII polymorphism [31].

In a Netherlands breast cancer study, 620 patients were examined for association between breast cancer mammographic density and XbaI and PvuII polymorphisms. The percentage density was higher in women with A genotypes (AA,AG) for XbaI polymorphism than in those with GG genotype. Also, the percentage density was higher in women with T genotypes (TT,TC) for PvuII polymorphism than those with CC genotype [18].

In our study there was no association between breast cancer and PvuII ,XbaI polymorphism which is consistent for PvuII polymorphism with many studies; South Korea, Sweden, US white and African American, Caucasian and Norway studies. (Table 9.)

However, two haplotypes in our study were significantly associated with breast cancer, A-G-T-C haplotype, increase the

Table 9. Previous studies on relation between breast cancer and PvuII and XbaI SNPs of ER-α.

Country	PvuII SNP	XbaI SNP	Number of patients	References
South Korea	No association	G allele protective A allele as a risk	205	Shin et al. 2003
Sweden	No association	No association	1556	Wedren et al. 2004
China	T allele is significantly associated	A allele is weakly associated	1069	Cai et al. 2003
Norway	No association	G allele protective A allele as a risk	360	Andersen et al. 1994
African-American and white-American	No association	No association	220	Vandevord et al. 2006
Caucasian	No association	G allele as a protective allele	393	Wang et al. 2007
Netherlands	T allele lead to increase in mammographic density	A allele lead to increase in mammographic density	620	Franzel et al. 2005
Jordan (our study)	No association	No association	100	(our study)

risk of breast cancer, and A-A-T-C haplotype, as a protective haplotype.

Our Jordanian study is For XbaI polymorphism, most of the studies (China, Norway, Netherlands...etc.) reported that A allele is a

risk allele for breast cancer and G allele as a protective allele for breast cancer. For our Jordanian study, XbaI polymorphism reported to be a nonsignificant polymorphism, but we have two limitations in our study. First, the number of samples obtained from patients used in our study was 100 samples, while other studies obtained more than 200 samples. China research, obtained 1069 breast cancer samples. As the number of samples increase, the distribution of population genotypes become more accurate. The second limitation, for Hardy-Weinberg equilibrium. XbaI patients were out of Hardy-Weinberg expectation, so the results we obtained from XbaI SNP may not be accurate and need further studies.

Although, we didn't find strong relation between our studied SNPs and the incidence of breast cancer like many studies [29,31], other studies showed a relation between breast cancer and these SNPs, however, these studies are not fully consistent with each other [26,18,24,27-28]. So the overall studies strongly suggest that the PvuII and XbaI polymorphisms have a possible relation with the incidence of breast cancer. Further studies of this region of ER-α gene and its association with breast cancer is needed.

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