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Evaluation of genetic polymorphism in estrogen receptor α gene as breast cancer risk.

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Abstract

Breast cancer is very common cancer worldwide. Estrogen is one of the most important steroid hormones which play an important role in breast cancer. The aim of this study was to evaluate frequency distribution of *ER-α* gene SNPs in breast cancer patients and controls to find out any association with breast cancer risk. In the present study, two hundred breast cancer patients and one hundred age matched controls were taken to study breast cancer risk factors. The odd ratio (ORs) at 95% confidence interval (CIs) was computed to study significance of risk factor on menopausal status. Contribution of *ESR1* gene in breast cancer was examined that allele frequency showed non-significant difference among patients and control group for rs138724640 and rs373404617 (p=0.4015 and p=0.8422 respectively). Odd ratio analysis showed that SNP rs2234693 and rs9340799 located on intron 1 were associated with breast cancer (p=0.0111, 0.025 respectively). SNP rs2077647 located on exon 3 showed only borderline significance (p=0.0777). C allele was found to be more prevalent in both patients and control group of rs138724640 and rs2234693. 'A' allele was more prevalent in breast cancer patients and controls of rs9340799 and in controls of rs373404617. The frequency of G allele was found to be more in breast cancer patients of rs373404617. In cases of SNP rs2077647, T allele was more frequent in both breast cancer patients and control group. Present study results suggest the difference of genotype distribution of *ESR1* SNPs between breast cancer patients and controls; hence establish the association with breast cancer risk. Further validation studies are required on additional *ESR1* SNPs to study the breast cancer risk using larger cohort.

Keywords: Breast carcinoma, Genotype, *ESR1*, Single nucleotide polymorphism (SNPs).

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Introduction

Estrogen is one of the most important steroid hormones which plays important role in breast growth and development of hormone dependent breast cancer [1]. Estrogen gives the activation or inhibition signals to estrogen responsive gene transcription and gene products expression [2]. There are several mechanisms by which cellular changes occur induced by estrogens.

The most important estrogens action is its binding to the specific receptor. Estrogens diffuse into the cell and binds to estrogen receptor (ER) of nucleus. This complex binds to estrogen response element sequences through protein-protein interactions with activator or specificity protein sites in the promoter region of estrogen responsive genes. It results in recruitment of co regulatory proteins to the promoter thus increase or decrease in mRNA levels hence a physiological response [3].

There are two main subtypes of estrogen receptors (ER), ER α and β. These subtypes possess a considerable homology in the DNA binding and ligand binding domain, but ER β is smaller than ER α and they diverge at transcriptional activation domain, indicating that they may target the distinct set of genes [4]. The distribution of both receptors differs in different tissues.

The ER α (*ESR1*) gene have molecular size 140 kb with eight exons encoded for protein encompasses of 595 amino acids with a molecular weight of 66 kDa. The promoter region and first intron of the gene usually contains a larger number of regulatory elements than other introns. Several sequence variations or single nucleotide polymorphisms (SNPs) in the *ERα* gene have been identified and found to be associated with either an increased or a decreased risk of various diseases. The most characterized SNPs of *ESR1* are *PvuII* and *XbaI* restriction site polymorphisms, located in the first intron [5].

The polymorphisms, *PvuII* and *XbaI* are 397 bp and 351 bp upstream of exon 2 and have been described by their reference ID numbers, rs2234693 and rs9340799, respectively [6]. Possible functional mechanisms attributed to these polymorphisms include a change in *ERα* gene expression by

alteration in the transcription factors binding and influence on alternative splicing of *ER-α* gene [7].

The molecular mechanisms by which the polymorphisms influence receptor activity are yet unclear. *PvuII* and *XbaI* RFLPs lie in an intronic which is non-functional area of the gene. This would be expected that other polymorphic areas of *ESR1*gene near by promoter may be associated with breast cancer risk. The human *ER-α* gene exhibits low mutational frequency in breast cancer tissue. However, the allelic variants have been found to be associated with breast cancer risk in many populations [8,9].

Present study aimed to evaluate frequency distribution of *ER-α* gene SNPs (rs138724640 (Taq1) exist in exon 1, SNPs rs2234693 (*PvuII*) and rs9340799 (*XbaI*) exist in intron 1, SNPs rs373404617 (*BstUI*) and rs2077647 (*SfaNI*) exist in exon 3) in breast cancer patients and control group to find out any association with breast cancer risk.

Materials and Methods

Study subject

The present study has been done on two hundred breast cancer female patients from Department of Radiotherapy, Pt. B.D. Sharma University of Health Sciences Rohtak. One hundred healthy female of about matched age group as those of breast cancer patients, with no history of breast cancer were selected for control group. Under complete aseptic conditions, 5 ml of venous blood was collected in sterile EDTA treated tubes (Bekton Dickenson, OK) from breast cancer patients. All samples were taken after institutional ethical committee permissions and personal consent of the patients or guardians (Registration no. PHY13-1009/12.11.13).

DNA extraction

The DNA was extracted from peripheral blood by using rapid improvised isolation of mammalian DNA method [10]. The purity was checked spectrophotometrically. Purity of DNA sample was checked by OD at OD_{260}/OD_{280}. The DNA prepared from blood was 20-50 Kb in size and suitable for use as a template in PCRs. The yield of DNA varies 5-15 µg per 300 µl of whole blood.

PCR-RFLP

Genotyping of estrogen receptor-α (*ESR1*) polymorphisms was done by amplification of genomic regions containing the polymorphisms with set of primers designed by this study (Table 1). PCR was performed by a Simpli Amp thermal cycler (Applied Biosystem, life technologies). The reactions mixture of 25 µL solution contained 1 µM of each primer, 10 ng of genomic DNA, 2.5 mM dNTPs, 25 mM $MgCl_2$ and 1.5 unit of Taq DNA polymerase (Applied Bio system) in 10X Taq buffer without $MgCl_2$ provided by the manufacturer.

Amplification process was done on an initial denaturation temperature 94°C for 3 min, followed by 35 cycles at 94°C for 45 s, annealing (Table 1) for 30 s and 72°C for 2 min, and a final extension at 72°C for 7 min. The final PCR product contains intron 1, exon 1 and 3 of the estrogen receptor-α (*ESR1*). The PCR product was subjected to restriction endonuclease digestion (Thermo Scientific) using Taq1, PvuII, XbaI, BstUI, and SfaN1 enzymes.

SNP rs138724640 (Taq1) genotypes were denoted by TT, TC, CC.Taq1 restriction produces 122 bp and 76 bp fragments. SNPs rs2234693 (PvuII) and XbaI polymorphisms genotypes were denoted by TT, TC, CC and AA, AG, GG respectively. Restriction digestion by PvuII produces two fragments of 936 and 436 bp while restriction digestion by XbaI enzyme produces 982 and 390 bp fragments. SNPs rs373404617 (BstUI) and rs2077647 (SfaNI) genotypes were denoted by GG, GA, AA and CC, CT, TT respectively. Restriction by BstUI produces fragments of 85 bp and 55 bp while restriction by SfaNI produces fragments of 131 bp and 9 bp (Figure 1).

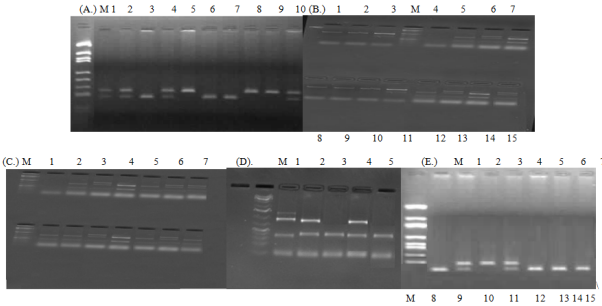

Figure 1. *Representative results of ESR1 SNPs restriction digestion (A.) rs138724640 restriction fragments (bp); Genotype: TT Lanes 5, 8, 9. TC lanes; 1, 2, 4, 10. CC lanes 3, 6, 7. (B.) rs2234693 restriction fragments (bp); Genotype: TT Lanes 1, 2, 3, 8, 9, 10. TC lanes 5, 6, 7, 14. CC lanes 11, 12, 15. (C.) rs9340799 restriction fragments (bp); Genotype: AA Lanes 6, 8, 9. AG lanes 4, 10, 11. GG lanes 2, 3, 5, 6, 7, 13, 14, 15. (D.) rs37340461 restriction fragments (bp); Genotype: GG Lanes 3, 5. GA lanes 2, 4. AA lanes 1. (E.) rs2077647 restriction fragments (bp); Genotype: CC Lanes 3. CT lanes 2, 4.TT lanes 1, 5, 6, 7. (Agarose conc.-1.5%, voltage-100 V, time-30 min).*

Statistical analysis

Statistical analysis was performed using Medcalc (version 17.2), SPSS software package version 22.0 (IBM Corp., Armonk, NY, USA) and Microsoft excel [11]. Results were expressed in number and percentage. Chi square test, odds ratio with 95% confidence level and z score estimation were performed in order to measure the association between *ER-* gene polymorphisms and breast cancer risk. The level of statistical significance was set at $P<0.05$.

Results

In the present study, *ESR1* gene polymorphism was examined in breast cancer patients and controls (Table 2). In SNP rs138724640 (Taq1), maximum breast cancer patients and controls were found in heterozygous condition. The TT (wild type) genotype frequency was lower both in breast cancer cases and controls. In SNP rs2234693 (PvuII), the CC (mutant) genotype frequency was lower in breast cancer patients and TT

(wild type) genotype in control group. Overall majority of participants of both groups were in heterozygous condition. In case of SNP rs9340799 (XbaI) GG (mutant) frequency was found lower and AG frequency higher in both groups.

In SNP rs373404617 (BstUI) frequency of AA (mutant) genotype was found to be low in breast cancer patients and GG (wild type) genotype in controls. Maximum number of breast cancer patients and controls were heterozygous. In case of SNP rs2077647 (SfaNI) CC (mutant) genotype was lower in both breast cancer patients and controls. Majority of breast cancer patients were found with CT genotype, whereas TT (wild type) genotype was in maximum number in control group.

The genotype analysis showed no statistically significant difference among cases and controls for rs138724640 and rs373404617 (p=0.4015 and p=0.8422 respectively). Odd ratio analysis showed that SNP rs2234693 and rs9340799 located on intron one were associated with breast cancer (p=0.0111, 0.025 respectively). SNP rs2077647 located on exon three showed only borderline significance (p=0.0777) (Table 2).

Allele frequency was counted in both breast cancer patients and controls for all SNPs. C allele was found to be more prevalent in both cases and controls of rs138724640 and rs2234693. A allele was more prevalent in breast cancer patients and controls of rs9340799 and in controls of rs373404617. The frequency of G allele was maximum in breast cancer patients of rs373404617. In cases of SNP rs2077647, T allele was more frequent in both breast cancer patients and controls.

Upon combining the rs2234693 and rs9340799 polymorphism nine haplotypes were made to study their association with breast cancer. AGTC haplotype showed statistically significant association with breast cancer risk (p=0.0004); whereas GGTT haplotype showed significant protection from breast cancer (p=0.0201). The other haplotypes were showed no statistically significant association with breast cancer risk (Table 3).

Table 1. *PCR oligonucleotide primers of estrogen receptor-α (ESR1) gene used in this study.*

SNP site	Primer site (5'-3')	Annealing temperature (°C)	Restriction enzyme
Intron1	f-5'-ctgccaccctatctgtatcttttcctattctcc-3'	65	PvuII
	r-5'-tctttctctgccaccctggcgtcgattatctga-3'		
Intron 1	f-5'-ctgccaccctatctgtatcttttcctattctcc-3'	65	XbaI
	r-5'-tctttctctgccaccctggcgtcgattatctga-3'		
Exon 1	f-5'-atatttccttgcccctgctt-3'	51	Taq polymerase I
	r-5'-gccctctgcctcagctaaat-3'		
Exon 3	f-5'-aaatcgagttgtgcctggag-3'	55	SfaNI
	r-5'-tccaggtagtagggcacctg-3'		
Exon 3	f-5'-aaatcgagttgtgcctggag-3'	55	BstUI
	r-5'-tccaggtagtagggcacctg-3'		

Table 2. *Genotype and allele frequency of ESR1 SNPs in patients and control group.*

SNP genotype	Patients	Controls	Or[a]	95% CI[b]	Z score	p	P[c]
rs138724640							
TT	60 (30%)	20%	1.7143	0.8946 to 3.2851	1.624	0.1043	0.4015
TC	71 (36%)	54%	0.4792	0.2718 to 0.8447	2.544	0.011	
CC	69 (34%)	26%	2.688	1.4067 to 5.1366	2.993	0.0028	
T allele	96 (47.52%)	94 (47%)					
C allele	106 (52.48%)	106 (53%)	0.8871	0.5133 to 1.5330	0.429	0.6678	
rs2234693							
TT	72 (36%)	21%	2.1161	1.1258 to 3.9776	2.328	0.0199	0.0111
TC	74 (37%)	41%	0.8451	0.4785 to 1.492	0.58	0.5621	

CC	54 (27%)	38%	0.6035	0.3317 to 1.0977	1.655	0.0980	
T allele	109 (49.55%)	83 (41.5%)					
C allele	91 50.45%)	117 (58.5%)	1.3863	0.7908 to 2.4304	1.14	0.2541	
rs9340799							
AA	80 (40%)	23%	0.7115	0.3666 to 1.381	1.006	0.3145	0.025
AG	96 (48%)	57%	1.8102	1.0181 to 3.2185	2.021	0.0433	
GG	24 (12%)	20%	0.5455	0.2508 to 1.1865	1.529	0.1263	
A allele	128 (64%)	102 (51%)					
G allele	72 (36%)	98 (49%)	1.041	0.5972 to 1.8148	0.142	0.8873	
rs373404617							
GG	52 (26%)	20%	1.4054	0.7241 to 2.7277	1.006	0.3145	0.8422
GA	98 (49%)	47%	1.0834	0.6220 to 1.8871	0.283	0.7771	
AA	50 (25%)	33%	0.6768	0.3658 to 1.2522	1.244	0.2136	
G allele	101 (50.5%)	87 (43.5%)					
A allele	99 (49.5%)	113 (56.5%)	1.3289	0.7594 to 2.3256	0.996	0.3193	
rs2077647							
TT	66 (33%)	42%	0.6802	0.3825 to 1.2096	1.312	1.312	0.0777
CT	70 (35%)	39%	0.8422	0.4740 to 1.4963	0.586	0.5581	
CC	64 (32%)	19%	2.0062	1.0444 to 3.8538	2.09	0.036	
C allele	99 (49.5%)	77 (38.5%)					
T allele	101 (50.5%)	123 (61.5%)	1.5732	0.8941 to 2.7681	1.572	0.1161	

OR[a]: Odds Ratio; CL[b]: 95% Confidence Interval; P[c]: Chi square value.

Table 3. *Comparison of rs2234693 and rs9340799 Haplotypes among cases and controls.*

Haplotype	Breast cancer cases	Controls	OR	95% CI	z statistic	Significance level (p)
AATT	11 (5%)	11 (11%)	0.6053	0.2887 to 1.2687	1.33	0.1836
AGTT	33 (17%)	13 (13%)	1.1606	0.8189 to 1.6449	0.837	0.4024
AATC	31 (15%)	11 (11%)	1.181	0.8218 to 1.6971	0.899	0.3685
AGTC	93 (46%)	23 (23%)	1.6173	1.2421 to 2.1057	3.57	0.0004
AGCC	7 (4%)	10 (10%)	0.375	0.1136 to 1.2384	1.609	0.1076
GGTT	7 (4%)	14 (14%)	0.256	0.0812 to 0.8073	2.325	0.0201
GGTC	9 (5%)	9 (9%)	0.5322	0.1718 to 1.6481	1.094	0.2741
GGCC	5 (2%)	4 (4%)	0.4898	0.0877 to 2.7369	0.813	0.4162
AACC	4 (2%)	5 (5%)	0.4162	0.0734 to 2.0474	1.116	0.2645

Significance level, p<0.05 OR: Odd Ratio; 95% CI: 95% Confidential Interval.

Discussion

The prognostic and therapeutic implication of estrogen receptor in breast cancer patients is recognized now days. Estrogen receptor (ER) status is an important predictive and prognostic factor in breast cancer [1]. Estrogen receptor expression has been considered to be present in two thirds of breast cancers [2].

There are many case control studies that have been conducted over the past decade on the different populations including Americans, Chinese, Brazil, Korean, Netherlanders, Indian and

Swedish [3,7,9]. There is variability in results of these studies. PvuII and XbaI polymorphism are the most studied variants for their possible role in breast cancer. Many studies found no significant association of PvuII polymorphism (rs2234693) with breast cancer [3,9]. Some studies found statistically significant association between PvuII polymorphism (rs2234693) and breast cancer [7,12].

In case of XbaI polymorphism (rs9340799), significant breast cancer risk association was found in many studies [13,14]. However, some studies found no association with breast cancer. The present study showed statistically significant association for both PvuII polymorphism (rs2234693) and XbaI polymorphism (rs9340799) similar to other studies [9,15]. SNP SfaNI (rs2077647) located on exon 3 showed only borderline significance (p=0.0777). The genotype analysis showed no statistically significant difference among cases and controls for rs138724640 (Taq1) and rs373404617 (BstUI) similar to other studies [16,17]. These polymorphic variants are associated with catalytic activity in conversion of estrogen to 4-hydroxy estrogens and DNA damage [18].

The susceptibility of breast cancer variants may be absent in one population than others. The frequencies may vary among different populations. These variants may not have the same effect among all populations due to different interactions of the variants with other genes or environmental factors [19]. It has been observed that allele frequency of a variant may be different among populations; the variant which are common in one group may not be frequent in the others.

In the present study, C alleles of SNP rs138724640 (53%) and T alleles of SNP rs2234693 (49%) were found more prevalent in breast cancer cases similar to other studies [7,12,20]. The T → C transition create a binding site for b-myb transcription factors, which results in lower expression of T allele of *ESR1* gene [21]. In this study, A allele was found in 64% breast cancer cases of SNP rs9340799. In controls 56% women noted to have A allele of SNP rs373404617 similar to other studies [7,22,23]. The frequency of G allele in breast cancer cases was 51% of rs373404617. In SNP rs2077647, the frequency of C allele in breast cancer cases was 50% similar to a study [24].

In the present study, the impact of combination of rs2234693 and rs9340799 polymorphism was investigated to study breast cancer risk association. Nine haplotypes were made upon combination of the rs2234693 and rs9340799 polymorphism. AGTC haplotype showed significant association with breast cancer risk; whereas GGTT haplotype showed significant protection from breast cancer (p=0.0201) similar to other studies [3,9].

Conclusion

In the present study, the genotype distribution of five *ESR1* SNPs (rs138724640, rs2234693, rs9340799, rs373404617 and rs2077647) was examined in breast cancer patients and controls to study the contribution of *ESR1* SNPs in occurrence of disease. The genotype analysis showed that SNP rs2234693 and rs9340799 showed significant association with breast cancer. SNP rs2077647 showed only borderline significance. Further keeping in view of the above information validation studies are required on additional *ESR1* SNPs to study the breast cancer risk. The prognostic and therapeutic value of research work would be examined and validated further on larger number of samples.

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References

1. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arc Pathol Lab Med 2010; 134: 48-72.
2. Osborne C, Schiff R. Mechanisms of endocrine resistance in breast cancer. Ann Rev Med 2011; 62: 233-247.
3. Alsheyab F, Dannoun A, Awad D, Mosameh Y. Association between estrogen receptor alpha polymorphisms and breast cancer risk in Jordanian Women. J Appl Biol Sci 2012; 6.
4. Mosselman S, Polman J, Dijkema R. ERβ: identification and characterization of a novel human estrogen receptor. FEBS Lett 1996; 392: 49-53.
5. Tang L, Chen L, Qi M, Su Y, Su F. Effects of passive smoking on breast cancer risk in pre/post-menopausal women as modified by polymorphisms of PARP1 and ESR1. Gene 2013; 524: 84-89.
6. Fu M. Breast cancer-related lymphedema: symptoms, diagnosis, risk reduction, and management. World J Clin Oncol 2014; 5: 241.
7. Madeira KP, Daltoe RD, Sirtoli GM, Carvalho AA, Rangel LB, Silva IV. Estrogen receptor alpha (ERS1) SNPs c454-397T>C (PvuII) and c454-351A>G (XbaI) are risk biomarkers for breast cancer development. Mol Biol Rep 2014; 41: 5459-5466.
8. Araujo KL, Rezende LC, Souza LS, Daltoe RD, Madeira KP, Paes MF, Herkenhoff FL, Rangel LB, Silva IV. Prevalence of estrogen receptor alpha PvuII (c454-397T>C) and XbaI (c454A>G) polymorphisms in a population of Brazilian women. Brazil Arc Biol Technol 2011; 54: 1151-1158.
9. Ramalhinho AC, Marques J, Fonseca-Moutinho JA, Breitenfeld L. Genetic polymorphims of estrogen receptor alpha-397 PvuII (T>C) and 351 XbaI (A>G) in a portuguese population: prevalence and relation with breast cancer susceptibility. Mol Biol Rep 2013; 40: 5093-5103.
10. Sambrook J, Russell DW. Isolation of high-molecular-weight DNA from mammalian cells using formamide. Cold Spring Harbor Protocols 2006; 2006: 3225.

11. Anghel A, Narita D, Seclaman E, Popovici E, Anghel M, Tamas L. Estrogen receptor alpha polymorphisms and the risk of malignancies. Pathol Oncol Res 2010; 16: 485-496.
12. Chattopadhyay S, Siddiqui S, Akhtar MS, Najm MZ, Deo SV, Shukla NK, Husain SA. Genetic polymorphisms of ESR1, ESR2, CYP17A1, and CYP19A1 and the risk of breast cancer: a case control study from North India. Tumor Biol 2014; 35: 4517-4527.
13. Shin A, Kang D, Nishio H, Lee MJ, Park SK, Kim SU, Noh DY, Choe KJ, Ahn SH, Hirvonen A, Kim JH. Estrogen receptor alpha gene polymorphisms and breast cancer risk. Breast Cancer Res Treat 2003; 80: 127-131.
14. Wang PH. Role of sex hormone receptors in ovulation. Taiwan J Obstetr Gynecol 2005; 44: 16-25.
15. Surekha D, Vishnupriya S, Rao DN, Sailaja K, Raghunadharao D. PvuII polymorphism of estrogen receptor-α gene in breast cancer. Ind J Human Gene 2007; 13: 97.
16. Rylander-Rudqvist T, Wedren S, Granath F, Humphreys K, Ahlberg S. Cytochrome P450 1B1 gene polymorphisms and postmenopausal breast cancer risk. Carcinogenesis 2003; 24: 1533-1539.
17. Wen W, Cai Q, Shu X, Cheng J, Parl F. Cytochrome P450 1B1 and catechol-O-methyltransferase genetic polymorphisms and breast cancer risk in Chinese women: results from the shanghai breast cancer study and a meta-analysis. Cancer Epidemiol Biomark Prev 2005; 14: 329-335.
18. Hanna I, Dawling S, Roodi N, Guengerich F, Parl F. Cytochrome P450 1B1 (CYP1B1) pharmacogenetics: association of polymorphisms with functional differences in estrogen hydroxylation activity. Cancer Res 2010; 60: 3440-3444.
19. Bamshad M. Genetic influences on health: does race matter? J Am Med Assoc 2005; 294: 937-946.
20. Ladd AG, Vasquez AA, Rivadeneira F, Siemes C, Hofman A, Stricker BC, Pols HA, Uitterlinden AG, van Duijn CM. Estrogen receptor α polymorphisms and postmenopausal breast cancer risk. Breast Cancer Res Treat 2008; 107: 415-419.
21. Herrington DM, Howard TD, Hawkins GA, Reboussin DM, Xu J, Zheng SL, Brosnihan KB, Meyers DA, Bleecker ER. Estrogen-receptor polymorphisms and effects of estrogen replacement on high-density lipoprotein cholesterol in women with coronary disease. N Engl J Med 2002; 346: 967-974.
22. van Duijnhoven FJ, Bezemer ID, Peeters PH, Roest M, Uitterlinden AG, Grobbee DE, Van Gils CH. Polymorphisms in the estrogen receptor α gene and mammographic density. Cancer Epidemiol Prev Biomarkers 2005; 14: 2655-2660.
23. Gail S, Prins S, Kenneth S, Korach. The role of estrogens and estrogen receptors in normal prostate growth and disease. Steroids 2008; 73: 233-244.
24. Gallicchio L, Berndt SI, McSorley MA, Newschaffer CJ, Thuita LW, Argani P, Hoffman SC, Helzlsouer KJ. Polymorphisms in estrogen-metabolizing and estrogen receptor genes and the risk of developing breast cancer among a cohort of women with benign breast disease. BMC Cancer 2006; 6: 173.

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