

# **Estrogen Receptor Alpha (ESR- $\alpha$ ) Gene Polymorphism Interactions with Intake of Contaminated Ground Water and Risk of Breast Cancer in North Indian Population**

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**ABSTRACT:** Epidemiological studies have shown association between consumption of contaminated drinking water and breast cancer risk. The aim of this study is to establish correlation and association of ESR- $\alpha$  polymorphism of PvuII and XbaI with consumption of contaminated ground water and breast cancer risk in North Indian population which was assessed by PCR-RFLP method. We conducted hospital-based case-control studies of 200 breast cancer patients with histologically confirmed three most prevalent type of breast cancer i.e Ductal Carcinoma In Situ (type 1 cancer), Invasive Breast Cancer (type 2 cancer), and Infiltrating Lobular Carcinoma (type 3 cancer), and matched controls from Mohan Dai Oswal Cancer Hospital, Ludhiana, Punjab and various parts of north India. We found strong consistent association between the ESR- $\alpha$  PvuII and XbaI polymorphism and breast cancer risk with consumption of untreated and contaminated ground water. The OR for genotype Pp was 1.0 (95%CI 0.58-1.80) suggesting increased in the risk of breast cancer with person having Pp genotype same is found in the women with type 1 and type 3 cancer. The decrease in OR (0.3) showed to be more significant in pp genotype and type 1 and type 2 cancer. Women who had lifetime consumed contaminated ground water had elevated frequencies of the pp genotype (41.9%) as compared to non user (28.9% and p value was 0.040 (i.e p<0.05). The p value (p<0.001) was found to be highly significant for the women who had not undergone hormone replacement therapy that in case of controls whereas the pp genotype (40.2%) and p allele frequencies were increased in patients using contraceptive as compare to nonusers. In case of passive smokers Pp genotype frequency was found to be higher as compare to nonsmokers and p value is found to be 0.002\*\* i.e is (p<0.01) that means non smokers have less risk of having breast cancer. Some protective role of use of multivitamin was found as Pp genotype frequency was lower and p value <0.007. In ESR- $\alpha$  XbaI polymorphism, there was low prevalence of xx genotype thus no role of this genotype was found in breast cancer. The OR for genotype Xx+xx genotype was 0.5 (95% CI: 0.29-0.80) and in type 1 and type 2 cancer were 0.5 (95% CI 0.29-0.93) and 0.5 (95% CI 0.22-0.92) respectively and found to be significant as p-value 0.003 and for type 1 and type 2 cancer, p-value 0.027 and p-value 0.025 respectively. The study thus highlighted the influence and significant association of ESR- $\alpha$  PvuII polymorphism and with drinking of contaminated ground water on the development and progression of breast cancer. Our finding shows significant less risk of having breast cancer in women who are nonsmokers, uses multivitamin, and who had done breast feeding.

**KEYWORDS:** Breast cancer, Menarche, Menopausal status, Polymorphism, HRT, ESR- $\alpha$

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## I. INTRODUCTION

Drinking water contaminated by waste water from industrial effluent, commercial products and excreted natural and pharmaceutical hormones is potential risk for Breast cancer [1,2-4]. Excessive use of pesticides; phenolic compounds in some detergents and plastics; and parabens in some cosmetics and prepared foods usually got mix with ground water and have been shown in animal and cell studies to mimic estrogen, which is an established breast cancer risk factor [2,3,5]. Mammary carcinogens include benzene and other organic solvents; polycyclic aromatic hydrocarbons (PAHs), which are products of combustion; some pesticides; and some pharmaceuticals and endogenous hormones (e.g., estrogen) [3,5-7]. Estrogen is a steroidal hormone that influences many physiological processes, which include female reproduction, cardiovascular control, and bone integrity. Due to its lipophilic characteristic, estrogen diffuses through plasmatic membrane and binds to its receptor (ER), member of the nuclear receptor superfamily, located in the nucleus and cytoplasm, forming a estrogen/ER complex. Two main iso forms of ER (called ESR- $\alpha$  and ESR- $\beta$ ) are known, which are encoded by separate genes. ESR- $\alpha$  gene is located on chromosome 6q25.1 whereas ESR- $\beta$  is located on chromosome 14q22-24 [8]. ESR- $\alpha$  gene encompass 140kb of DNA composed by eight exons, encoding a 595 amino acids protein with a molecular weight of about 66 kDa. The first intron of a gene, like the promoter, usually contains a larger number of regulatory sequences than other introns. Several single nucleotide polymorphisms (SNPs) have been identified on estrogen receptor and some of them were associated with either an increased or a decreased risk of various diseases [9,10] The best characterized SNPs of ESR- $\alpha$  are the c454-397T>C and c454A>G site polymorphisms, both located in the first intron. These polymorphisms are 397 and 351 bp upstream of exon 2 and have been described by the name of detecting restriction enzyme, PvuII and XbaI, and their reference ID numbers are rs2234693 and rs9340799, respectively [11].

The PvuII and XbaI SNPs of the ESR- $\alpha$  gene were found to be associated previously with various pathological conditions, including cardiovascular disorders [12], venous thromboembolism [13], miscarriage [14], and severe pre-eclampsia [15]. Also, several estrogen-dependent characteristics such as the onset of menopause [16], lumbar spine bone mineral density (BMD), vertebral bone area and vertebral fracture risk in post-menopausal women [17], as well as waist circumference [18], blood pressure [19], coronary reactivity [20] and lipid profile [21] have been described. The transcriptional regulation of ER $\alpha$  is poorly understood and only a small number of regulatory regions have been well characterized [22].

A possible functional mechanism attributed to PvuII and XbaI polymorphisms includes a change of ESR- $\alpha$  gene expression by altering the binding of transcription factors. We conducted a case-control study to investigate whether drinking of contaminated deep dig ground drinking water contaminated by waste water from industrial effluent or pesticides or unknown factors and other life style factors increases the risk of breast cancer by influencing single nucleotide polymorphism in ESR- $\alpha$  XbaI genotype and ESR- $\alpha$  PvuII genotype.

## II. MATERIALS AND METHODS

Histologically confirmed 200 breast cancer patients and matching 200 controls were recruited from Mohan Dai Oswal Cancer Hospital, Ludhiana, Punjab. None of the patients had received radiation or chemotherapy. Epidemiological history, such as age at onset of breast cancer, age at menarche, age at menopause, diet, reproductive history, family history, number of abortion, breast feeding, use of hormone replacement therapy (HRT), use of multivitamin, smoking status, use of contaminated ground water, was taken through a personal interview with the breast cancer patients and matching controls using a specific proforma. The patients were screened for type of the breast cancer and related data was collected with the help of an oncologist.

Women were identified as smokers if they had life time history of smoking more than one hundred cigarettes. Exposure to passive smoking was assessed by questioning spouses smoking history. Data related to consumption of contaminated drinking water was taken into consideration if the women had life time history of drinking water from hand pumps, digging deep in ground and from open ponds from more than 10 years without giving them any treatment like reverse osmosis, UV filtration or simple filtration. Genotyping was successfully done for 400 women for the PvuII polymorphism and 400 for the XbaI polymorphism. Informed consent was obtained from all participants on a structured questionnaire.

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### III. POLYMORPHISM ANALYSIS

Genomic DNA was isolated by SDS/proteinase K and phenol chloroform method (<http://genome-ou-edu/protocols-book/protocol-partII.html>). We selected two single nucleotide polymorphisms (SNPs) in the estrogen receptor alpha gene (ESR- $\alpha$ ) i.e PvuII and XbaI polymorphisms which were analyzed by polymerase chain reaction restriction fragment lengths polymorphism (PCR-RFLP) [23]. A 109kb DNA fragment - that contained two polymorphic sites - was amplified using forward and reverse primers 5' CTGTGTTGTCCATCACTTCATC 3' and 5' CCATTAGAGACCAATGCTCATC 3'. PCR was performed through 30 cycles by the following steps: denaturation at 95°C for 60 s; annealing at 52°C for 30 s; and extension at 72°C for 30 s. PCR products were digested with the restriction endonucleases PvuII and XbaI (Invitrogen, Carlsbad, CA, USA). Digested products were run onto 2% agarose gel electrophoresis. Heterozygous Pp genotype exhibited 119, 78 and 41 bp lengths fragment and heterozygous Xx genotype exhibited fragments of 119, 88 and 31 bp lengths. Capital P or X represent the absence of restriction site while lower-case p or x indicate the presence of restriction site.

### IV. STATISTICAL ANALYSIS

Mean values were presented with their standard deviation (SD) and assessed by Students' *t*-test and standard tests were employed to determine the significance of differences in allelic and genotypic distributions between cases and controls. Multivariate logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CI) after adjustment for age for estimating the associations between the SNPs in ESR- $\alpha$  XbaI and PvuII and the risk of breast cancer. The Hardy-Weinberg Equilibrium (H-WE) was tested by a goodness-of-fit test to compare the allele and genotype proportions in case and control subjects. For all the tests, a two-tailed probability value (*P* value) of less than 0.05 was considered as statistically significant. All the data was analysed by SPSS 19.0 (IBM SPSS, Chicago, USA).

### V. RESULTS

Two hundred breast cancer patients and two hundred healthy controls were analyzed for genotype distribution of PvuII and Xba I polymorphism of the ESR- $\alpha$  gene. Characteristics of the study population were compared by case-control status, as shown in Table I. The mean age of controls ((47.5  $\pm$  10.7years) was significantly lower (*p* < 0.01) than that of breast cancer patients (50.6  $\pm$  9.4Years). The mean age at diagnosis of breast cancer in the present sample was 50.6  $\pm$  9.4 years. The genotype distribution was studied with respect to risk confounding factors, such as menopausal status, age at menarche, age at first pregnancy, number of pregnancies, breast feeding, family history of breast cancer, use of hormone replacement therapy, use of contraceptive pills, smoking status, use of multivitamins, use of contaminated ground water and three main types of breast cancer and were found to be significantly different in cases and controls. In our study breast feeding showed tendency for an association with decreased breast cancer risk, and this association found to be significant (Table 1). Age at first child, age at first menstruation, and age at menopause were significantly associated with breast cancer risk (Table 1)[37,38].

**Table 1**  
**Selected Characteristics of Study Population**

Characteristics	Cases	Controls	<i>p</i> -Value
The <i>p</i> -value for the breast feeding, oral contraceptive, HRT, use of contaminated ground water and use of multivitamin was calculated by Fisher's exact test on the 2 $\times$ 3 contingency table.			
Age, <i>n</i> (mean $\pm$ SD)	(50.6 $\pm$ 9.4)	(47.5 $\pm$ 10.7)	0.003
Age at menarche, <i>n</i> (mean $\pm$ SD)	(13.8 $\pm$ 1.6)	496 (15.1 $\pm$ 1.6)	
Age at menopause, <i>n</i> (mean $\pm$ SD)	99 (49.5 $\pm$ 3.8)	85 (47.2 $\pm$ 3.3)	0.000
Age at 1st pregnancy, <i>n</i> (mean $\pm$ SD)	425 (26.6 $\pm$ 5.4)	331 (22.2 $\pm$ 3.5)	0.000
<b>Pregnancy (<i>n</i> =400), <i>n</i> (%)</b>			
No	145 (25.4)	166 (33.4)	0.000
Yes	142 (71.0)	186 (93.0)	

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<b>Breast feeding (n = 400), n (%)</b>			
No	58 (29.0)	14 (7.0)	0.000
Yes	142 (71.0)	186(93.0)	
<b>Oral contraceptive use (n = 400), n (%)</b>			
No	73(59.5)	119 (59.5)	0.000#
Yes	127 (63.5.)	81 (40.5)	
<b>Menopausal status (n = 400), n (%)</b>			
Premenopausal	64 (32.0)	98 (49.0)	0.000***
Postmenopausal	136 (42.6)	102 (36.3)	
<b>Use of HRT</b>			
No	63(31.0)	178(79.0)	0.000#
Yes	138(69.0)	22(11.0)	
<b>Tobacco smoking (n = 400), n (%)</b>			
Non smoker	59 (29.5)	134 (67.0)	0.000
Passive smoker	128 (64.0)	61 (30.5)	
Active smoker	13 (6.5)	6(2.5)	0.013
	62 (11.3)	33 (6.7)	
<b>Intake of multivitamin (n = 400), n (%)</b>			
No	113 (56.5)	108 (54.0)	0.18
Yes	87 (43.5)	92(46.0)	0.688
<b>Family history of breast cancer in first-degree relatives (n = 400), n (%)</b>			
No	135(67.5)	175(87.5)	0.026
I degree	54 (27.0)	19 (9.5)	
2 <sup>nd</sup> degree	11(5.5)	6(3.0)	
<b>Consumption of contaminated ground water</b>			
No	45 (22.5)	157 (78.5)	0.000
Yes	155(77.5)	43 (21.5)	

Genotyping analysis

# Fisher's exact test

\* p<0.05

\*\* p< 0.01

\*\*\* p<0.001(highly significant)

Table 2 and Table 3 shows the genotype frequencies of the ESR- $\alpha$  gene polymorphisms in PvuII and XbaI in both breast cancer patients and controls. The frequency of XbaI genotype in the population was XX 71.5% (n=143), Xx 18.5% (n=37), xx 10.0% (n=20) and in type 1 cancer it was found to be XX 71.1% (n=87), Xx 18.5% (n=22), xx 8.4% (n=10) and type 2 cancer it was found to be XX 70.3% (n=45), Xx 17.2% (n=11), xx 12.5% (n=8) and least for type 3 cancer (Table 2). In XbaI polymorphism, there was low prevalence of xx genotype thus no role of this genotype was found in any three types of breast cancer (Table 2).

**TABLE 2**  
**Distribution of ESR- $\alpha$  XbaI genotype among cases and controls**

	N	XX	Xx	Xx	$\chi^2$ , df, p-value
<b>Controls</b>	200	168(84.0)	25(12.5)	7(3.5)	10.591, 2, 0.005**
<b>Cases</b>	200	143(71.5)	37(18.5)	20(10.0)	
<b>Type 1</b>	119	87(73.1)	22(18.5)	10(8.4)	1.214, 4, 0.876
<b>Type 2</b>	64	45(70.3)	11(17.2)	8(12.5)	
<b>Type 3</b>	17	11(64.7)	4(23.5)	2(11.8)	

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In the population genotyped for *PvuII*, this polymorphism was distributed as follows: PP 16.0% (n=32), Pp in 45.0% (n=90), pp 39.0% (n=78) and in type 1 cancer it is found that PP16.0% (n=16) Pp42.0%(n= 50) , pp in 42.0% (n=50). In type 2 cancer genotype PP15.6% (n=10) Pp48.4%(n=31), pp in 35.9% (n=23) and least frequency was found in third type of cancer (Table 3).

**TABLE 3**  
**Distribution of ESR- $\alpha$  PvuII genotype among cases and controls**

	N	PP	Pp	pp	$\chi^2$ , df, p-value
<b>Controls</b>	200	43(21.5)	124(62.0)	33(16.5)	25.258, 2, 0.000**
<b>Cases</b>	200	32(16.0)	90(45.0)	78(39.0)	
<b>Type 1</b>	119	19(16.0)	50(42.0)	50(42.0)	1.509, 4, 0.825
<b>Type 2</b>	64	10(15.6)	31(48.4)	23(35.9)	
<b>Type 3</b>	17	3(17.6)	9(52.9)	5(29.4)	

The frequency of the Pp genotype (45%) was lower than that of controls (62%) and in type 1 cancer it was found to be 42%, and increased in case of type 2 cancer (48%) and highest in type 3 cancer with a corresponding increase in P allele frequency ( $\chi^2 = 25.258; P < 0.01$ ). The OR for genotype Xx+xx genotype was 0.5(95% CI:0.29-0.80) and in type 1 and type 2 cancer were 0.5(95% CI 0.29-0.93) and 0.5(95% CI 0.22-0.92) respectively (Table 4) and found to be significant as p value is p-value 0.003 and for type 1 and type 2 cancer p-value 0.027 and p-value 0.025 respectively (Table 4).

**TABLE 4**

**Odds ratio and corresponding 95% confidence interval(CI) of ESR- $\alpha$  XbaI genotypes and risk of cancer**

Genotypes	All OR(n-200)	Type 1	Type 2	Type 3
<b>ESR-<math>\alpha</math> XbaI</b>				
<b>XX</b>	1.0(Ref.)	1.0	1.0	1.0
<b>Xx</b>	0.6(0.32-1.04)	0.6(0.30-1.16)	0.6(0.26-1.43)	0.4(0.11-1.66)
<b>Xx</b>	0.3(0.11-0.77) p-value 0.009	0.4(0.12-1.08)	0.2(0.07-0.76) p-value 0.008	0.2(0.04-1.81)
<b>Xx+xx</b>	0.5(0.29-0.80) p-value 0.003	0.5(0.29-0.93) p-value 0.027	0.5(0.22-0.92) p-value 0.025	0.4(0.11-1.15)

The OR for genotype Pp was 1.0(95%CI 0.58-1.80) suggesting increased in the risk of breast cancer with person having Pp genotype and same is found in the women with type 1 and type 3 cancer (Table 5). The decrease in OR (0.3) showed to be more significant in pp genotype and in type 1 and type 2 cancer (Table 5).

**TABLE 5**

**Odds ratio and corresponding 95% confidence interval (CI) of ESR PvuII genotypes and risk of cancer**

Genotypes	All OR(n-200)	Type 1	Type 2	Type 3
<b>ESR PvuII</b>				
<b>PP</b>	1.0(Ref.)	1.0	1.0	1.0
<b>Pp</b>	1.0(0.58-1.80)	1.1(0.55-2.15)	0.9(0.39-2.18)	1.0(0.20-4.13)
<b>pp</b>	0.3(0.16-0.61) p-value 0.000	0.3(0.14-0.62) p-value 0.000	0.3(0.13-0.86) p-value 0.020	0.5(0.08-2.44)
<b>Pp+pp</b>	0.7(0.41-1.19)	0.7(0.37-1.31)	0.7(0.30-1.51)	0.8(0.17-3.10)

No other association of ESR XbaI polymorphism was found with any risk factor (Table 6,7,8,9,10). On assessing the interaction between ESR- $\alpha$  XbaI polymorphism and use of contraceptives in cases and controls, the XX genotype was found to be highest i.e 69.3% whereas frequency of Xx genotype (17.3%) was slightly higher than xx genotype(13.4%) (Table 6).

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**TABLE 6**  
**Assessment of interaction between ESR- $\alpha$  XbaI and use of contraceptives in cases and controls**

ESR- $\alpha$ XbaI	Users		Non-users	
	Cases (n-127)	Controls (n-81)	Cases (n-73)	Controls (n-119)
<b>XX</b>	88(69.3)	66(81.5)	55(75.3)	102(85.7)
<b>Xx</b>	22(17.3)	10(12.3)	15(20.5)	15(12.6)
<b>xx</b>	17(13.4)	5(6.2)	3(4.1)	2(1.7)
$\chi^2$ , df, p-value	4.222, 2, 0.121		3.447, 2, 0.178	

Table 7 shows the interaction between ESR- $\alpha$  XbaI polymorphism and use of HRT in cases and controls. No significant p value was observed as XX genotype accounts for 69.6% whereas it was 18.8% for Xx genotype and only 11.6% for xx genotype in patients having breast cancer.

**TABLE 7**  
**Assessment of interaction between ESR- $\alpha$  XbaI and use of HRT in cases and controls**

ESR- $\alpha$ XbaI	Users		Non-users	
	Cases (n-138)	Controls (n-22)	Cases (n-62)	Controls (n-178)
<b>XX</b>	96(69.6)	21(95.5)	47(75.8)	147(82.6)
<b>Xx</b>	26(18.8)	1(4.5)	11(17.7)	24(13.5)
<b>xx</b>	16(11.6)	0(0.0)	4(6.5)	7(3.9)
$\chi^2$ , df, p-value	6.588, 2, 0.037*		1.470, 2, 0.480	

The XX genotype (72.9%) and X genotype frequency were less in patients using contaminated drinking water as compare to controls (93.0%). No association of any kind was found in ESR- $\alpha$  XbaI polymorphism and use contaminated drinking water in cases and controls (Table8).

**TABLE 8**  
**Assessment of interaction between ESR- $\alpha$  XbaI and use of contaminated groundwater in cases and controls**

ESR- $\alpha$ XbaI	Users		Non-users	
	Cases (n-155)	Controls (n-43)	Cases (n-45)	Controls (n-157)
<b>XX</b>	113(72.9)	40(93.0)	30(66.7)	128(81.5)
<b>Xx</b>	25(16.1)	2(4.7)	12(26.7)	23(14.6)
<b>xx</b>	17(11.0)	1(2.3)	3(6.7)	6(3.8)
$\chi^2$ , df, p-value	7.781, 2, 0.020*		4.538, 2, 0.103	

On studying the interaction between ESR- $\alpha$  XbaI polymorphism and smoking status in cases and controls (Table 9), no association of any kind was found in risk of getting breast cancer and status of active smoking or passive smoking (Table 9).

**TABLE 9**  
**Assessment of interaction between ESR- $\alpha$  XbaI and smoking status in cases and controls**

ESR- $\alpha$ XbaI	Non-smoker		Passive smoker		Active Smoker	
	Cases (n-59)	Controls (n-134)	Cases (n-128)	Controls (n-61)	Cases (n-13)	Controls (n-5)
<b>XX</b>	45(76.3)	113(84.3)	87(68.0)	50(82.0)	11(84.6)	5(100.0)
<b>Xx</b>	8(13.6)	16(11.9)	28(21.9)	9(14.8)	1(7.7)	0(0.0)
<b>xx</b>	6(10.2)	5(3.7)	13(10.2)	2(3.3)	1(7.7)	0(0.0)
$\chi^2$ , df, p-value	3.390, 2, 0.184		4.649, 2, 0.098		0.865, 2, 0.649	

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No protective role of use of multivitamin was found on assessing the interactions between ESR- $\alpha$  XbaI polymorphism and use of multivitamins in cases and controls. No significant p value was found for Xx or xx genotype (Table10).

**TABLE 10**  
**Assessment of interaction between ESR- $\alpha$  XbaI and use of multivitamin in cases and controls**

ESR- $\alpha$ XbaI	Users		Non-users	
	Cases (n-87)	Controls (n-92)	Cases (n-113)	Controls (n-108)
<b>XX</b>	58(66.7)	79(85.9)	85(75.2)	89(82.4)
<b>Xx</b>	18(20.7)	10(10.9)	19(16.8)	15(13.9)
<b>Xx</b>	11(12.6)	3(3.3)	9(8.0)	4(3.7)
$\chi^2$ , df, p-value	9.944, 2, 0.007**		2.374, 2, 0.305	

The pp genotype (40.2%) and p allele frequencies were increased in patients using contraceptive as compare to nonusers (23.5%) (Table 11).P value was found be 0.030 (i.e p<0.05) means patients with pp genotype and who had used contraceptives are at higher risk of having breast cancer.

**TABLE 11**  
**Assessment of interaction between ESR- $\alpha$  PvuII and use of contraceptives in cases and controls**

ESR- $\alpha$ Pvu II	Users		Non-users	
	Cases (n-127)	Controls (n-81)	Cases (n-73)	Controls (n-119)
<b>PP</b>	21(16.5)	13(16.0)	11(15.1)	30(25.2)
<b>Pp</b>	55(43.3)	49(60.5)	35(47.9)	75(63.0)
<b>pp</b>	51(40.2)	19(23.5)	27(37.0)	14(11.8)
$\chi^2$ , df, p-value	7.028, 2, 0.030*		17.453, 2, 0.000***	

The p value (p<0.001) was found to be highly significant for the women who had not undergone hormone replacement therapy when compared among cases and controls. So we may predict from the Table 12 that women who undergo HRT therapy are at high risk of having breast cancer (Table12).

**TABLE 12**  
**Assessment of interaction between ESR- $\alpha$  PvuII and use of HRT in cases and controls**

ESR- $\alpha$ Pvu II	Users		Non-users	
	Cases (n-138)	Controls (n-22)	Cases (n-62)	Controls (n-178)
<b>PP</b>	25(18.1)	3(13.6)	7(11.3)	40(22.5)
<b>Pp</b>	63(45.7)	15(68.2)	27(43.5)	109(61.2)
<b>Pp</b>	50(36.2)	4(18.2)	28(45.2)	29(16.3)
$\chi^2$ , df, p-value	4.025, 2, 0.134		21.611, 2, 0.000***	

Women who had lifetime used contaminated drinking ground water had elevated frequencies of the pp genotype (41.9%) as compared to Non users (28.9%) and p value was found to be 0.040(i.e p<0.05) means patients with pp genotype have high risk of having breast cancer(Table 13).

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**TABLE 13**

**Assessment of interaction between ESR- $\alpha$  PvuII and use of contaminated ground water in cases and controls**

ESR- $\alpha$ PvuII	Users		Non-users	
	Cases (n-155)	Controls (n-43)	Cases (n-45)	Controls (n-157)
<b>PP</b>	24(15.5)	10(23.3)	8(17.8)	33(21.0)
<b>Pp</b>	66(42.6)	24(55.8)	24(53.3)	100(63.7)
<b>pp</b>	65(41.9)	9(20.9)	13(28.9)	24(15.3)
$\chi^2$ , df, p-value	6.455, 2, 0.040*		4.326, 2, 0.115	

In case of passive smokers Pp genotype frequency was found to be higher as compare to nonsmokers and p values found to be 0.002 i.e is ( $p < 0.01$ ) means non smokers have less risk of having breast cancer as compare to passive or active smokers (Table14).

**TABLE 14**

**Assessment of interaction between ESR- $\alpha$  PvuII and smoking status in cases and controls**

ESR- $\alpha$ PvuII	Non-smoker		Passive smoker		Active Smoker	
	Cases (n-59)	Controls (n-134)	Cases (n-128)	Controls (n-61)	Cases (n-13)	Controls (n-5)
<b>PP</b>	9(15.3)	30(22.4)	19(14.8)	13(21.3)	4(30.8)	0(0.0)
<b>Pp</b>	28(47.5)	84(62.7)	59(46.1)	36(59.0)	3(23.1)	4(80.0)
<b>pp</b>	22(37.3)	20(14.9)	50(39.1)	12(19.7)	6(46.2)	1(20.0)
$\chi^2$ , df, p-value	12.082, 2, 0.002**		7.128, 2, 0.028*		5.182, 2, 0.075	

Some protective role of use of multivitamin was found as Pp genotype frequency was lower and p value was found to be 0.007(i.e  $p < 0.01$ ) and significant (Table15).

**TABLE 15**

**Assessment of interaction between ESR- $\alpha$  PvuII and use of multivitamin in cases and controls**

ESR- $\alpha$ PvuII	Users		Non-users	
	Cases (n-87)	Controls (n-92)	Cases (n-113)	Controls (n-108)
<b>PP</b>	13(14.9)	17(18.5)	19(16.8)	26(24.1)
<b>Pp</b>	36(41.4)	55(59.8)	54(47.8)	69(63.9)
<b>pp</b>	38(43.7)	20(21.7)	40(35.4)	13(12.0)
$\chi^2$ , df, p-value	9.955, 2, 0.007**		16.568, 2, 0.000***	

### VI. DISCUSSION

In this study, the prevalence of PvuII polymorphism in P and p allele and XbaI polymorphism in X and x alleles in north Indian Population of adult women was evaluated in, similar to what was already done for other gene polymorphisms (23,24). Results for PvuII allele frequencies were similar to most of other studies around the world, although concerning the XbaI allele frequencies, the present data differed from the previous other studies [25,26,27,28,29,30, 31]. The main difference of the women inserted in this study was the prevalence of the allele x which was very low for the experimental population.

Present study shows significant increased in breast cancer risk for women with Pp genotype and tends to have increased risk of having type 1 and type 3cancer. Decreased risk of was found for women having pp genotype[31.] A significant association  $P < 0.040$  was found in women who were consuming contaminating untreated ground water[1,2,32,33] and were having contraceptives pills and undergone hormone replacement therapy also.(34). Use of multivitamin found to have some protective role in our study for ESR- $\alpha$  PvuII polymorphism in Pp genotype ( $p = 0.007$ ). In case of passive



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smokers Pp genotype frequency was found to be higher as compare to nonsmokers.(35,36) with  $p < 0.01$ . p value in case of non smokers were significant as compare to passive smokers. some protective role of use of multivitamin was found as Pp genotype frequency was lower and When the type of cancer was considered, both Pp and pp genotype frequencies of PvuII were possible markers for breast cancer. Moreove, ESR- $\alpha$  XbaI polymorphism due to low prevalence of xx genotype show no role of this genotype in breast cancer .But on combining OR for genotype Xx+xx genotype was found to be marginally increased risk for breast cancer and also show significant results in type 1 and type 2 cancer. No other association of ESR- $\alpha$  XbaI polymorphism was found with any other risk factor.

## VII. CONCLUSION

The study thus highlighted the influence and significant association of of ESR- $\alpha$  PvuII polymorphism with drinking of contaminated ground water and resulting in development and progression of breast cancer. Our finding shows significant less risk of having breast cancer in women who are nonsmokers, use multivitamins and who had done breast feeding.

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## REFERNCES

1. Brody,J.G., Aschengrau,A., McKelvey,W., Swartz, C. H.,Kennedy,T.,and Rudel,RA.,”Breast cancer risk and drinking water contaminated by wastewater: a case control study”, *Environmental Health: A Global Access Science Source*, Vol.5,pp.28, doi:10.1186/1476-069X-5-28, 2006.
2. Brody, J.G, Rudel, R.A., “Environmental pollutants and breast cancer”, *Environ Health Perspect* , Vol.111, no.8, pp.1007-1019, 2003.
3. Davis ,D.L, Bradlow ,HL, Wolff M.,Woodruff T, Howl ,DG.,Anton-Culver H., “Medical hypothesis: xenoestrogens as preventable causes of breast cance”, *Environmental Health Perspectives*,Vol.101, no.5, pp.372-377, 1993.
4. Wolff, M.S., Collman, G.W., Barret,t JC.,and Huff, J., “Breast cancer and environmental risk factors: epidemiological and experimental findings”, *Annual Review of Pharmacology and Toxicology* ,Vol.36, pp.573-596, 1996.
5. Soto, AM., Sonnenschein. C., Chung, KL, Fernandez ,MF.,Olea ,N.,and Serrano, FO., “The E-SCREEN assay as a tool to identify estrogens: An update on estrogenic environmental pollutants”,*Environmental Health Perspectives* , Vol.103, Suppl 7, pp.113-122, 1995.
6. Jensen, E.V.,and Jacobsen, H.I., “Basic guides to the mechanism of estrogen action”,*Rec Prog Horm Res.*,Vol. 18, pp.387-414, 1962.
7. Nilsson, S.,Makela, S.,Treuter, E.,and Tujague, M., “Mechanisms of estrogen action”, *Physiol Rev*, Vol.81, pp.1535-1565, 2001.
8. Kuiper, G.G.J.M.,Carlsson, B., Grandien, K. et al., “Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptor  $\alpha$  and  $\beta$ ”, *Endocrinology*,Vol.138, pp.863-70, 1997.
9. Yoshidome ,K.,, Shibara ,M.A., Couldrey, C.,Korach ,K.S.,and Green, J.E., “Estrogen promotes mammary tumor development in C3(1)/SV40 large T-antigen transgenic mice: paradoxical loss of estrogen receptor alpha expression during tumor progression”, *Cancer Res*,Vol. 60, pp.6901-6010, 2000.
10. Gennari, L.,Merlotti ,D., and De Paola V., “Estrogen receptor gene polymorphisms and the genetic of osteoporosis: a huge review”, *American Journal of Epidemiology*, Vol. 161, pp.307-320, 2005 .
11. Hill, S.M., Fuqua, S.A.,Chamness, G.C., Greene, G.L.,McGuire ,W.L., “Estrogen receptor expression in human breast cancer associated with an estrogen receptor gene restriction fragment length polymorphism”,*Cancer Research*,Vol 49, pp.145-148, 1989.
12. Lawlor, D.A., Timpson ,N., and Ebrahim, S., “The association of oestrogen receptor alpha-haplotypes with cardiovascular risk factors in the British Women’s Heart and Health Study.”, *European Heart Journal*, Vol.27, pp.1597-1604, 2006.
13. Straczek,C., Alhenc-Gelas, M., Aubry ,M.L, and Scarabin, P.Y., “On behalf of the Estrogen and Thromboembolism Risk (ESTHER) study Group. Genetic variation at the estrogen receptor a locus in relation to venous thromboembolism risk among postmenopausal women.”, *J Thromb Haemost.*, Vol. 3, pp.1535-1537, 2005.
14. Silva, I.V., Rezende, L.C.D., Lane., and S.P., “Evaluation of PvuII and XbaI polymorphisms in the estrogen receptor alpha gene (ESR1) in relation to menstrual cycle timing and reproductive parameters in post-menopausal women”,*Maturitas*,Vol. 53, pp.213-220, 2010.
15. Molvarec, A.,Ver. A., and Fekete ,A.,, “Association between estrogen receptor  $\alpha$  (ESR1) gene polymorphisms and severe preeclampsia.”, *Hypertens Res.*, Vol.3, pp.205-11, 2007.
16. Weel, A.E., Uitterlinden, A.G.,Westendorp, I.C., Burger, H.,Schuit ,S.C.,and Hofman A., “Estrogen receptor polymorphism predicts the onset of natural and surgical menopause”, *J Clin Endocrinol Metab.*, Vol. 84, pp.3146-3150, 1999.
17. Van,Meurs.,J.B.,Schuit ,S.C.,Weel, A.E., Van der Klift, M.,Bergink ,A.P., “Association of 5’ estrogen receptor alpha gene polymorphisms with bone mineral density, vertebral bone area and fracture risk”, *Hum Mol Genet.*, Vol.12, pp.1745-1754,2003.
18. Fox, C.S.,Yang, Q., Cupples, L.A.,et al., “Sex-specific association between estrogen receptor-alpha gene variation and measures of adiposity: the Framingham Heart Study”, *J Clin Endocrinol Metab.*, Vol.90,pp. 6257-6262, 2005.

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(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 6, June 2016

19. Peter ,I.,Shearman ,A.M.,and Zucker, D.R.,”Variation in estrogen-related genes and cross-sectional and longitudinal blood pressure in the Framingham Heart Study.”, *J Hypertens.*,Vol. 23, pp.2193-2200, 2005.
20. Lehtimaki, T., Laaksonen, R., andMattila, K.M.,, “Oestrogen receptor gene variation is a determinant of coronary reactivity in healthy young men”, *Eur J Clin Invest.*, Vol.32, pp. 400-404., 2002.
21. Molvarec, A.,Nagy ,B.,Kovács, M.,Walentin, S.,Mreh, E.,Rigó Jr., J., Szalay, and J.,Füst G., “Lipid, haemostatic and inflammatory variables in relation to the estrogen receptor  $\alpha$  (ESR1) PvuII and XbaI gene polymorphisms.”,*Clin Chim Acta.*, Vol.380, pp.157-164.,2007.
22. Penolazzi, L, Lambertini, E.,Aguari, G, del., Senno ,L, and Piva, R., “Cis element 'decoy' against the upstream promoter of the human estrogen receptor gene”, *Biochim Biophys Acta.* Vol. 1492, pp.560-567, 2000 .
23. Bittencourt-Oliveira, M.C., Cunha, M.C.C., Moura ,and A.N.M., “Genetic Polymorphism in Brazilian Microcystis spp. (Cyanobacteria) Toxic and Non-Toxic Through RFLP-PCR of the cpcBA-IGS”, *Braz Arch Biol Technol.*,Vol. 54, no.4, pp.901-909, 2009.
24. Lopes, P.A., Gaspar, G.,Napoleão, P., and Santos, M.C., “Comparison of Apolipoprotein E Polymorphism and other Atherosclerotic Risk Factors in two Portuguese Populations - the Influence of Insularity”, *Braz Arch Biol Technol.*,Vol. 50,no.5, pp.803-813, 2007.
25. Sakimiuk ,A.J.,Malgorzata, N., Bogusiewicz ,M.,Adamiak ,A.,Skorupski ,P.,Miotla, P., Rechberger ,T.,and Haczyński J., “Prevalence of estrogen receptor  $\alpha$  PvuII and XbaI polymorphism in population of Polish postmenopausal women”, *Folia histochem cytobiol.*, Vol. 45, pp.331-338, 2007.
26. Gennari, L., Merlotti ,and D.,De Paola ,V., “Estrogen receptor gene polymorphisms and the genetic of osteoporosis: a huge review”, *Am J Epidemiol.*,Vol. 161, pp.307-320, 2005.
27. Becherini ,L., Gennari ,L., Masi ,L.,Mansani, R.,Massart ,F.,Morelli ,A.,et al., “Evidence of a linkage disequilibrium between polymorphisms in the human estrogen receptor alpha gene ant their relationship to bone mass variation in postmenopausal Italian women”, *Hum Mol Genet.*, Vol.9, pp.2043-2050, 2000.
28. Long, J.R., Xu ,H., Zhao, L.J.,Liu, P.Y.,Shen ,H.,and Liu,Y.J .,The oestrogen receptor a gene is linked and/or associated with age of menarche in different ethnic groups. *J Med Genet.*, Vol. 42, pp.796-800, 2005.
29. Shearman, A.M., Cupples, L.A., Demissie, S.,Peter, I., Schmid, C.H., Karas, R.H...et al. Association Between Estrogen  $\alpha$  Receptor Gene Variation and Cardiovascular Disease”, *JAMA.*, Vol.290, pp.2263-2270, 2003.
30. Demissie,S.,Cupples, L.A., Shearman, A.M.,Gruenthal, K.M.,Peter, I., and Schmid, C.H., “Estrogen receptor- $\alpha$  variants are associated with lipoprotein size distribution and particle levels in women: The Framingham Heart Study”, *Atherosclerosis*, Vol.185,210-218.,2006.
31. Shen, Y.,Li ,Dk., Wu ,J., Zhang, Z.,Gao, E., Joint effects of *CYP1A1 Msp1*, “ERapIha *PvuII*,and ERapIha *XbaI* polymorphism on the risk of breast cancer:results from a population – based case control study in shanghai ,china”, *Cancer Epidemiol Biomarker Prev* ,Vol.15,pp 342-347, 2006.
32. Gallagher,L.G., Vieira,V.M .,Ozonoff, D., Webster,T.F.,and Aschengrau,A., “Risk of breast cancer following exposure to tetrachloroethylene-contaminated drinking water in Cape Cod, Massachusetts: reanalysis of a case-control study using a modified exposure assessment”, *Environmental Health* ,Vol. 10,no.47 ,doi:10.1186/1476-069X-10-47, 2011.
33. Peter, W.,Weyer, Ccerhan, J,R Burton cKross, George R.Hallberg:et al. “Municipal Drinking Water Nitrate Level and Cancer Risk in Older Women:The Iowa Womens Health Study”.,*Epidemiology* ,Vol.11 pp.No 3, 2001.
34. Taeko Mizoo,Naruto Taira,Keik, Nishiyama,Takayuki Iwamoto,et al. “Effects of life style and single nucleotide polymorphism in Breast cancer risk: a case control study in japanese women”, *BMC cancer*,Vol.13,pp.565, 2013.
35. Gauder, M.M.,Gapstur, S.M.,Sun, J.,Diver,W.R., Hannnan, L.M.,Thun ,M.J., “Active Smoking and Breast Cancer Risk :original cohort data and meta anaysis” *j natl cancer inst .*, Vol.105,pp.515-525, 2013.
36. Nagata, C.,Mizoue ,T.,Tanaka, K.,Tsuji ,I.,Wakai, K.,Inoue ,M.,Tsugane, S., “Research Group for the Development and Evaluation of Cancer Prevention Strategies in Japan. Tobacco Smoking and Breast Cancer Risk:an evaluation based on systematic review of epidemiological evidence in Japanese population” *Jpn J Clin Oncol.*,Vol.36,no.6,pp.387-94, 2006.
37. Mcpherson, K.,,Steel, C.M.,Dixon ,J.M., “ABC of Breast diseases .Breast Cancer epidemiology ,risk factors and genetics”, *BMJ* ,Vol.321,pp. 624-628, 2003.
38. Negata, C.,Hu Yh.,Shimizu., “Effects of menstrual and reproductive factors on the risk of breast cancer: meta analysis of case -control study in japan” *Jpn J Cancer Res.*, Vol.86, pp.910-915, 1995.