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).

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

Accepted on December 28, 2018
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 Kbp in size and suitable for use as a template in PCR. 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 MgCl₂ and 1.5 unit of Taq DNA polymerase (Applied Bio system) in 10X Taq buffer without MgCl₂ 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 SfaNI 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 392 and 390 bp fragments. SNPs rs373404617 (BstUI) and rs2077647 (SfaNI) genotypes were denoted by GG, GA 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).

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...
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(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.

<table>
<thead>
<tr>
<th>SNP site</th>
<th>Primer site (5'-3')</th>
<th>Annealing temperature (°C)</th>
<th>Restriction enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intron 1</td>
<td>f-5’-tctccctctggtatccattcttccc-3’</td>
<td>65</td>
<td>PvuII</td>
</tr>
<tr>
<td></td>
<td>r-5’-ttttctcctggtatccattcttccc-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intron 1</td>
<td>f-5’-tctccctctggtatccattcttccc-3’</td>
<td>65</td>
<td>XbaI</td>
</tr>
<tr>
<td></td>
<td>r-5’-ttttctcctggtatccattcttccc-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 1</td>
<td>f-5’-atattctctgccacccttgctt-3’</td>
<td>51</td>
<td>Taq polymerase I</td>
</tr>
<tr>
<td></td>
<td>r-5’-gcctcctgctcactaagtgta-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 3</td>
<td>f-5’-aatctcgtgtgctcgtggag-3’</td>
<td>55</td>
<td>SfaNI</td>
</tr>
<tr>
<td></td>
<td>r-5’-tcacggtaaggggacactcgt-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 3</td>
<td>f-5’-aatctcgtgtgctcgtggag-3’</td>
<td>55</td>
<td>BstUI</td>
</tr>
<tr>
<td></td>
<td>r-5’-tcacggtaaggggacactcgt-3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

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

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

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...
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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 (TaqI) 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 association with breast cancer risk; whereas GGTT haplotype showed significant association with breast cancer risk association. Nine haplotypes were made upon combination of PvuII 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 (TaqI) 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].

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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.

Acknowledgment

Authors are grateful to acknowledge the M.D. University Rohtak and Pt. B.D. Sharma University of Health Sciences Rohtak for providing the help and support.

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