Estrogen and Vitamin D Receptors Genes

ESTROGEN AND VITAMIN D RECEPTORS GENES POLYMORPHISMS IN BREAST CANCER WOMEN
Magdy M. Ibrahim; Wael H. Elsawy*, Samy H.M. Ibrahim and Marwa H.S. Hussein
Medical Biochemistry and Clinical Oncology* Departments, Faculty of Medicine, Zagazig University

ABSTRACT
Background: Estrogen receptor (ER) and vitamin D receptor (VDR) is a ligand-activated transcription factor that mediates estrogen and vitamin D actions in target tissues. Several common polymorphisms of the ER-α gene and VDR gene have been reported to be associated with alterations in receptor expression and function. Objective: We evaluated the hypothesis that genetic polymorphisms in the PvuII restriction site of ER-α gene and FokI restriction site of VDR may be associated with breast cancer risk in Egyptian. Methods: In this study the involvement of two RFLPs, one at the ER-α gene locus, denoted as PvuII and the other at the VDR gene as denoted as FokI in breast cancer were examined in 50 breast cancer cases and 50 age frequency-matched controls. A case-control comparison was performed and the genotype distributions examined according to different tumor and population parameters. Result(s): PvuII polymorphism was associated with an increased risk of breast cancer (OR = 1.9 (0.75 5.0), P=0.01), while there was no significant difference in genotype frequency of the FokI polymorphism between controls and cases. In addition, significant association was found in patients with LN metastasis carrying the ER –α PvuII polymorphism. Also this study showed non-significant but generally higher relative risk of breast cancer for the f allele carriers of FokI polymorphism of Vitamin D receptors gene with breast cancer risk (OR =2.65(1.5- 5.2)  P=0.000). We did not find significant association between ERs gene PvuII restriction polymorphism and VDRs gene FokI polymorphism however there is significant association between the f allele and the p allele in cases (P=0.02). Conclusion(s): These results suggest that biomarkers for genetic polymorphisms could be used for the identification of breast cancer risk among Egyptian women.
Keywords: Breast cancer, estrogen receptor, polymorphism, vitamin d receptor

INTRODUCTION
Breast cancer is a common malignant disease in females and its incidence is increasing worldwide. It accounts for 22% of all female cancers. The estimated annual incidence of breast cancer worldwide is about one million case(1).

There are several well-established risk factors for breast cancer and a variety of others currently under study(2) such as family history, breast cancer susceptibility genes which are divided into 2 categories: ‘the high-risk’ breast cancer susceptibility genes including BRCA1, BRCA2, PTEN, TP53, LKB1/STK11 and CDH1 and the ‘low to moderate-risk’ breast cancer susceptibility genes CHEK2, TGFβ1, CASP8 and ATM genes(3).

Other factors implicated are mammographic density, ionizing radiation, histology of benign lesions, diet, environmental agents, cigarette smoking, and estrogen replacement therapy(2).

Estrogen plays a central role in the normal development of the mammary gland but is also involved in breast cancer progression(3).

Genetic variation in genes involved in estrogen synthesis, metabolism and signal transduction have been suggested to play a role in breast carcinoma. Estrogen exerts its biological effect through binding to estrogen receptors (ERs)(4), localized at the cytosolic and nuclear level. These receptors belong to the nuclear receptor hormone superfamily and are ligand-inducible transcription factors. There are two types of estrogen receptors, the estrogen receptor-α (ER-α) and the estrogen receptor-β (ER- β).

John et al.(5) have found that vitamin D intake and serum concentrations of vitamin D metabolites inversely associated with risk for developing BC. 1,25(OH)2D, the biologically most active form of vitamin D, exerts its antiproliferative effects and modulate transcription of genes involved in cellular differentiation and cell growth by binding to vitamin D receptor (VDR). VDR is expressed in most cell types, including normal and malignant breast tissues.

The VDR gene is located on the long arm of chromosome 12 (12q12-14). It consists of at least five promoter regions, eight protein-coding exons, and six untranslated exons which are alternatively spliced. The Fok1 restriction enzyme identifies a polymorphic site in exon 2 at the 50 end of the VDR gene(6).

Many subsequent molecular epidemiological studies were performed to assess the associations of VDR polymorphisms including Fok1, Bsm1, Apa1, and Taq1 with BC risk.
However, the results were different or even contradictory. Single study may not have enough statistical power to detect dose-response relationships or even overall effects. Given the amount of accumulated data, a quantitative synthesis of the evidence was deemed important. In this article, we reported results from a case control study that examined the association of polymorphisms ER-α PvuII res. and VDR FokI res. with the risk of breast cancer

**Patients and Methods**

Patients’ Recruitment and Samples Collection: A case group of 50 breast cancer female patients were recruited consecutively for this study: The breast cancer patients were diagnosed at the Department of Oncology, Zagazig University, Egypt. The diagnosis was confirmed by histologic examination of malignant breast tissue. Detailed information on cancer diagnosis and treatment, including, tumor size, tumor grade, presence or absence of lymph node involvement was obtained from medical records of these patients. The control group was randomly selected from the female general population with no history of any type of cancer. Controls were confirmed to be free from breast cancer by physical examination and mammography. Potential participants were approached. Those who consented were interviewed. All study subjects completed a structured questionnaire during an in person interview. A structured questionnaire was used to obtain information about age, body mass index, tobacco smoking habits, menstrual and reproductive history (including menopausal status-age at menarche, parity, age at full term pregnancy and use of oral contraceptives) pathological diagnosis, age at diagnosis and family history of breast cancer in first and second degree relatives/history of benign breast mass, history of diabetes mellitus and hypertension. Written informed consent was obtained from all tested subjects, and the study protocol was approved by relevant committees for the use of human subjects in research.

**DNA extraction:**

Blood was collected in a heparinized vacuum tube from patients as well as controls. Genomic DNA was extracted from buffy coat fractions using the BioBasic DNA isolation kit (Bio Basic Inc. Ontario-Canada) following the manufacturer’s protocol. Genotypic assay for Polymorphisms of both the estrogen receptor α Gene PvuII restriction site and VDR FokI restriction site. ERα and VDR genotyping were determined with PCR-RFLP. The DNA samples were all genotyped for the PvuII and fokI restriction enzyme polymorphisms. The RFLPs were coded as P-p (PvuII) and F-f (FokI )upper case lettering signifying the absence, and the lower case lettering, the presence of the restriction site. For the PvuII of ERs The oligonucleotide primers utilized for our analysis were forward 5’-CTGCCACCTATCTGTATCTTTT-3’and reverse-5’-ACCCCTGGCGTCCATTATCTGA-3’. The 50-µL reaction mixture contained 25 -µL of master mix (Promega), 0.2 mM of each primer. 5 µL of DNA eluate was used for the reaction. After an initial denaturation at 94 °C for 5 minutes, 30 cycles (94°C for 1 min, 50 °C for 1 min, and 72 °C for 90 seconds) were performed and were followed by a final 10 minute extension at 72 °C in a thermal cycler (Peqlab). The amplification products (1.3 Kbp) were visualized by staining with ethidium bromide after electrophoresis on 1% agarose gel. All sets of PCRs included negative control samples containing no template DNA) for avoidance of PCR contaminant artifacts. (Fig.1a). The PCR products which contained a part of intron 1 and exon 2 of the ESR1 gene, were digested with PvuII (Fermentas) restriction enzymes at 37°C for 22 hrs producing fragments of 1300 bp (C allele P) 450 + 850bp (T allele p) and of 130, respectively. The DNA fragments were then separated using 1.5% agarose gel and detected by ethidium bromide staining (Figs b & c). For the FokI restriction site of VDR The oligonucleotide primers and utilized for our analysis VDR2a: 5’-AGCTGGCCCCTGGCAGCTGCTCTGCTCT-3’ and VDR2b: 5’-ATGGAAACACCTTGCTTCTCCCTC-3’. PCR reaction was performed in a final volume of 25 µl that contained: 2X PCR Mix: 12.5 µl. And Primer mix (100 pM): 4 µl. Genomic DNA: 5 µl. And Deionized water: 3.5 µl.

The amplification was carried out using thermal cycler PTC-100 machine (MJ Research, Inc., Watertown, Mass. USA) The amplification conditions were 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec. A final elongation period of 5 min at 72°C was added after 30 cycles. PCR products were digested with 4 µL FokI and 5 µL New England Biolabs buffer (Beverly, MA) for 1.5 hr at 37°C and then electrophoresed through a 2% agarose gel for 2 hr at 80 V.

The f genotype was indicated by the presence of the restriction site that generates two fragments of 196 bp and 69 bp. The F genotype was indicated by a single uncleaved 265-bp fragment. Determination of VDR genotype FF, Ff,
or ff was indicated based on the FokI cleavage pattern. The DNA fragments were then separated using 1.5% agarose gel and detected by ethidium bromide staining. Statistic Analysis. Chi-squared (χ²) statistics were used to evaluate case control difference in the distribution of allele types and genotypes. Odds ratios (ORs) and 95% confidence intervals (CIs), were used to measure the strength of the association between ER-α and VDR genes polymorphisms and breast cancer risk.

**RESULTS**

The distributions of clinical characteristics of the collected breast cancer cases are shown in (table 1).

Case patients with breast cancer and control subjects were comparable in age (mean age for cases 53.3 ± 9.24 years [range:36 - 68]), while in controls the mean age is 54.60 ± 6.34 [range:44 - 65]) (table 2).

Allele frequencies of ER-α gene PvuII polymorphisms in the control group showed that the P allele was more prevalent among controls (0.55) than cases (0.36) in the PvuII polymorphism (table 3).

Approximately 38% of controls and 24% of cases were carrying PP genotype, while 40% of controls and 48% of cases were carrying Pp genotype and 36% of cases and 14% of controls were carrying pp genotype (table 4).

There was no significant difference in genotype of the Fok I polymorphism of VDR gene between controls and cases; however, Allele frequencies of VDR gene Fok1 polymorphisms in the control group showed that the f allele was more prevalent among cases (0.60) than controls (0.35) (table 5).

The association between FokI genotypes and PvuII genotypes in cases show no statistically significant relation between pp, PP, Ff, ff, of Fok1 restriction site of VDR gene polymorphism and PvuII restriction site of ERS gene polymorphism. While between Pp and Ff there was statistically significant relationship (table 6).

<table>
<thead>
<tr>
<th>Table (1): Characteristics of studied groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
</tr>
<tr>
<td>- Family history of breast cancer</td>
</tr>
<tr>
<td>- Contraception</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table (2): Age in different studied groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Mean± SD</td>
</tr>
<tr>
<td>Range</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table (3): Alleles of PvuII of ERS gene polymorphisms in cases and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele (N=100)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>C (P)</td>
</tr>
<tr>
<td>T (p)</td>
</tr>
</tbody>
</table>

*p< 0.05 when compared with control
Table (4): Genotypes of ERsgene polymorphisms in cancer breast patients and controls:

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>grouping</th>
<th>P-value</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (N=50)</td>
<td>Controls (N=50)</td>
<td>X²</td>
</tr>
<tr>
<td>(C/C)PP</td>
<td>Count 12</td>
<td>19</td>
<td>% 24.0% 38.0% 2.3</td>
</tr>
<tr>
<td>(C/T)Pp</td>
<td>Count 24</td>
<td>20</td>
<td>% 48.0% 40.0% 0.6</td>
</tr>
<tr>
<td>(T/T)p</td>
<td>Count 18</td>
<td>7</td>
<td>% 36.0% 14.0% 6.4</td>
</tr>
</tbody>
</table>

Table (5): Alleles of Fok1 of VDRgene in cases and controls:

<table>
<thead>
<tr>
<th>Allele of cases (N=100)</th>
<th>Allele of controls (N=100)</th>
<th>χ²</th>
<th>P-value</th>
<th>OR (95.0% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>F</td>
<td>60</td>
<td>60.0</td>
<td>35</td>
<td>35.0</td>
</tr>
<tr>
<td>F</td>
<td>40</td>
<td>40.0</td>
<td>65</td>
<td>65.0</td>
</tr>
</tbody>
</table>

*p< 0.05 when compared with control

Table (6): Association between VDRgene polymorphisms and ERS genes polymorphisms in cancer breast patients:

<table>
<thead>
<tr>
<th>Fok1</th>
<th>Count</th>
<th>χ²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PvuII</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T/T)Pp</td>
<td>Count</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>(C/T)Pp</td>
<td>%</td>
<td>77.8%</td>
<td>31.8%</td>
</tr>
<tr>
<td>(C/C)Pp</td>
<td>Count</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>(PvuII)</td>
<td>%</td>
<td>11.1%</td>
<td>68.2%</td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

DISCUSSION

Worldwide, breast cancer comprises 10.4% of all cancer incidence among women, making it the second most common type of non-skin cancer (after lung cancer) and the fifth most common cause of cancer death. In 2004, breast cancer caused 519,000 deaths worldwide (7% of cancer deaths; almost 1% of all deaths). In Egypt, it is the most common cancer among women, representing 18.9% of total cancer cases.

Most known risk factors for breast cancer can be linked to hazardous effects of hormonal exposures, although other risk factors such as female (1% male), aging, relative (mother or sister), menstrual history (early onset or late menopause), child birth after the age of 30, exogenous estrogen, radiation exposure and obesity are also relevant in some populations.

Approximately 15% of all breast cancer cases can be attributed to familial and genetic influences.

Estrogen is implicated in the development of breast cancer, based on data from both clinical and animal studies; risk factors associated with breast cancer reflect cumulative exposure of the breast epithelium to estrogen.

Two current hypotheses exist to explain this relationship. In the first, binding of estrogens to the ER stimulates proliferation of mammary...
cells, increasing the target cell number within the tissue, and the increase in cell division and DNA synthesis elevates the risk for replication errors, which may result in the acquisition of detrimental mutations that disrupt normal cellular processes such as apoptosis, cellular proliferation, or DNA repair\(^{(13)}\).

In the second hypothesis, estrogen metabolism leads to the production of genotoxic by-products that could directly damage DNA, again resulting in point mutations. There is evidence that estrogen may act through both mechanisms to initiate and/or promote mammary cancer\(^{(14)}\).

Currently, both SERMs and aromatase inhibitors are used in the treatment of breast cancer, and patients whose tumors are ER-positive respond well to these endocrine therapies. Further, both tamoxifen and raloxifene have proven effective as chemopreventive agents for breast cancer in high-risk women\(^{(15)}\).

The association of ER-genetic polymorphisms with breast cancer risk attracts much attention because ER functions as a hormone-dependent transcriptional regulator, which, in turn plays a significant role in the development of breast cancer\(^{(10)}\).

Several ER-gene polymorphisms have been reported. Different biomarkers mapping estrogen receptor alpha have been associated with breast cancer risk. The most studied variants in this gene are the single nucleotide polymorphisms (SNPs) PvuII (C/T where T and C allele are often reported as p and P allele, present in intron 1, 397\(^{(17)}\).

These variants have been implicated in gene expression by influencing transcription\(^{(18)}\).

Variation in the estrogen receptor gene PvuII has been associated with an increased risk of developing breast cancer\(^{(19)}\).

Previous studies investigating ER polymorphisms and breast cancer risk have produced mixed results, which may be explained by ethnicity of the populations under study. While some studies have found an increased risk for the p alleles of the PvuII polymorphisms, which agree with our study, others have found an increased risk only for the (P) allele\(^{(18, 20)}\).

In addition, other studies found no effect at all for either of these polymorphisms\(^{(14)}\). In the present study, we found evidence of an association between ER-\(\alpha\) PvuII polymorphism and breast cancer risk.

Allele frequencies of ER-gene PvuII polymorphisms in the control group were similar to those reported previously from other studies conducted in Asian populations\(^{(18, 20)}\).

Polymorphism at the PvuII restriction site (p allele) was associated with an elevated risk of breast cancer especially in young age. Two case studies showed that PvuII polymorphism was related to a younger age at breast cancer diagnosis\(^{(14)}\). We did not observe a similar association, while other studies\(^{(14, 18)}\) also did not find this association.

PvuII polymorphisms are located in intron of the ER-gene and are 50 bp apart\(^{(14)}\).

Yaich et al.\(^{(14)}\) examined the PvuII polymorphism in the tumor tissue of 257 primary breast cancer patients and 140 peripheral blood DNA samples from women without breast cancer. Breast cancer patients with a pp genotype were significantly younger than women with PP or Pp genotype at the time of cancer diagnosis. In our case-control study, we found that the polymorphism at the PvuII restriction site (p allele) was associated with an elevated risk of breast cancer but not with exact group of age.

It would be useful to study the interaction of the ER-gene with genes involved in these pathways. In summary, in this population-based case-control study, we found that PvuII polymorphism in the ER-gene was associated with breast cancer risk. Additional studies are needed to understand the nature of the association.

The first study on breast cancer association with VDR polymorphisms was conducted by Ruggiero et al. in 1998. They found those women who carried bb homozygous had almost a four times higher risk of developing metastatic breast cancer compared with BB genotype carriers. Many subsequent molecular epidemiological studies were performed to assess the associations of VDR polymorphisms including Fok1, Bsm1, Apa1, Taq1 with breast cancer risk. However, the results were different or even contradictory\(^{(21)}\).

In various observational studies, vitamin D intake and serum concentrations of vitamin D metabolites have been associated with decreased risk for developing breast cancer. Apart from the role that vitamin D plays in maintaining calcium homeostasis, its antiproliferative effects (by influencing cell differentiation, cell growth and apoptosis) are well established\(^{(22)}\).

Vitamin D from both diet and endogenous production is converted via two consecutive hydroxylation steps to 25-hydroxyvitamin D (25[OH]D) and to 1,25-dihydroxyvitamin D (1,25[OH]2 D). The biologically most active form of vitamin D is 1,25(OH)2D, which mainly exerts its antiproliferative effects by binding to the vitamin D receptor (VDR) and acting in complex as a transcriptional factor for a variety of genes,
including those involved in cell differentiation and cell growth.(23)

The VDR is present in a variety of cell types, including malignant and normal breast cells. Various studies have assessed associations between various polymorphisms in the VDR gene and breast cancer risk, with inconsistent results. These polymorphisms include three frequently analyzed, highly linked single nucleotide polymorphisms (SNPs) FokI, BsmI, ApaI and TaqI at the 3’ end of the VDR gene.(24)

13 papers have analysed the association of FokI with breast cancer risk (21, 25, 26, 27, 28, 29) are reporting non-significant but generally higher relative risk of breast cancer for the f allele carriers which agree with our study.

Three other studies(30, 31, 32) did not observe any association.

While Sinotte et al.(24); Chen et al.(33); Gapska et al.(34); Tang et al.(35) and Raimondi et al.(36) showed a statistically significant increase in relative risk of breast cancer in women with ff genotype compared with women with the FF genotype. Moreover, these studies suggest that relative risk of breast cancer increases with the increasing dosage of the f allele. If our observed associations are valid and causal, about 13 and 25% of the incidence rate of breast cancer in Ff and ff carrier would be attributable to their respective FokI genotypes.

The FokI polymorphism is located at exon 2 start codon of the VDR gene. Individuals designated F have VDR proteins missing three amino acids (424 amino acids) and those designated f have longer VDR proteins (427 amino acids) that have been shown to be functionally less efficient(37).

Since f allele results in a less efficient VDR, the increase in breast cancer incidence associated with f allele could possibly be overturned, or at least reduced by raising vitamin D levels, for instance through vitamin D supplementation. Interaction between VDR FokI SNP and vitamin D has been recently observed for prostate cancer.(38)

We did not find significant association between ERs gene PvuII res polymorphism and VDRs gene FokI res polymorphism however there is association between the f allele and the p allele in cases.

More studies should be done to examine the association between the polymorphisms in the steroid receptors genes especially in breast cancer patients.

CONCLUSION

Our study also showed a significant association between ER-α PvuII polymorphism and risk of cancer breast.

Also this study showed non-significant but generally higher relative risk of breast cancer for the f allele carriers of FokI with breast cancer risk.

We did not find significant association between ERs gene PvuII res polymorphism and VDRs gene FokI res polymorphism however there is association between the f allele and the p allele in cases.

REFERENCES


8- Park S, Koo J, Kim JH, Yang WI, Park BW and Lee KS. Clinicopathological characteristics of mucinous carcinoma of the breast in Korea: comparison with invasive ductal carcinoma-not otherwise
24- Sinotte M, Rousseau F, Ayotte P, Dewailly E and Diortio C. Vitamin D receptor polymorphisms (FokI, BsmI) and breast cancer risk: association replication in two case-control studies within French Canadian population. Endocrine-Related Cancer 2008; 15: 975–983.
28- McKay JD, McCullough ML, Ziegler RG, Kraft P and Saltzman BS. Vitamin D receptor polymorphisms and breast cancer


التباني الجنيني لكل من جيني مستقبلات الأستروجين وفيتامين د سرطان الثدي في النساء

الأستاذ الدكتور/ مجدي م. إبراهيم \* الأستاذ الدكتور/ وائل حسن الصاوي الأستاذ والدكتور/ سامي حسن م. إبراهيم و الطبيب/ مروه حسن سليمان حسن

قسم الكيمياء الحيوية الطبية و* قسم علاج الأورام

يعتبر سرطان الثدي أكثر أنواع الأورام الخبيثة انتشارا في السيدات، هناك العديد من العوامل التي يعتقد أنها تساعد على حدوث هذا النوع من الأورام، من هذه العوامل ما يتعلق بالعمر، الحالة الإنجابية، استخدام الأدوية التي تحتوي على الهرمونات، التاريخ العائلي، ونمط الحياة مثل نمط التغذية، ونظام النوم، والنشاط البدني، وعدد الاعضاء، وقد أثبتت بعض الدراسات أن هناك العديد من العوامل التي تؤثر على بعض الجينات، والتي يعتقد أنها قد تلعب دورًا في حدوث سرطانات الثدي، ومن هذه الجينات وجود تباين في شكل (تعدد شكلي) الجين الخاص بمستقبلات هرمون الاستروجين، و أيضا الجين الخاص بمستقبلات فيتامين (D).

الهدف من البحث: إيضاح دور التباين الجنيني لكل من الجين الخاص بمستقبلات فيتامين د ومستقبلات هرمون الاستروجين في التطور المرضي لحالات سرطان الثدي.

المرضي وطريق البحث: تمثل هذه الدراسة في قسم الكيمياء الحيوية والبيولوجيا الجزيئية والأورام بكلية الطب جامعة الزقازيق.

امتدت هذه الدراسة على 100 سيدة مسجّلة في مجموعتان:
- سوف يتم أخذ موافقه كتابية من المرضى لأخذ عينات الدم.

المجموعة الأولى: تشمل 50 سيدة أصحاء لا يعانون من سرطان الثدي كمجموعة ضابطة.
المجموعة الثانية: تشمل 50 سيدة يعانون من سرطان الثدي

نتيجة البحث:

تين من البحث وجود علاقة بين التهور الشكلي في الجين الخاص بمستقبلات هرمون الاستروجين، و بين حدوث سرطان الثدي، وإن وجود هذا التهور يزيد من فرص حدوث هذا المرض في حين أن لا يوجد علاقة واضحة بين التحور الشكلي في الجين الخاص بمستقبلات فيتامين د، و بين حدوث المرض.

تعد العديد من الدراسات السابقة بينت نفس النتائج في حين اختلقت دراسات أخرى بدلاً من هذه الدراسة لذلك المزيد من الدراسات مطلوبة لتأكيد هذه النتائج باستخدام وسائل أخرى، و العمل على تحولات أخرى في هذه الجينات، مع مرض سرطان الثدي، لوقفه على خلفية ثابتة بالنسبة لعلاقة هذا المرض بالتباني الجنيني في هذين الجينين.