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Assessment of the Relationship Between ERBB4 rs13393577 Polymorphism and Breast Cancer Susceptibility in Iranian Population

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Abstract

Background: Studies have shown that the polymorphism of genes involved in breast cancer in combination with environmental factors has an important role in the progression of breast cancer.
Objectives: In this study, the association between polymorphism of the ERBB4 gene with breast cancer was investigated.
Methods: In the present study, 110 patients with breast cancer and 110 healthy individuals were selected as controls. DNA extraction was performed on patients' samples. The tetra-ARMS-PCR method was used to study rs13393577 polymorphism. Finally, statistical analysis was performed using SPSS software using *t*-test.
Results: The results of the study in the patients' group showed that the frequencies of TT, CT, and CC genotypes were 73, 15, and 1.8%, and allelic frequency in this group for T and C alleles were 95 and 5%, respectively. In addition, the results of the study in the control group showed that the frequencies of TT, CT, and CC genotypes were 86, 11, and 0.9%, respectively. The allelic frequencies in the control group for the T and C alleles were 97 and 3%, respectively. In addition, the risk ratio and allelic reliability were obtained for T allele was OR: 3.06: and CI = 0.31 - 29.94 and for C allele was OR: 0.32: and CI: 0.03 - 3.19, respectively. Finally, statistical analysis showed that no significant difference was observed between the two groups ($P > 0.05$).
Conclusions: The results of the present study showed that rs13393577 polymorphism in the EGFR gene (ERBB4) is not a genetic predisposing factor for breast cancer.

Keywords: rs13393577, EGFR, ERBB4, Breast Cancer, Tetra-ARMS-PCR

1. Background

Cancer is a group of diseases that are caused by disorders in the process of cell proliferation. Breast cancer is a malignancy that develops in breast cells and is more than one hundred times more common in women than in men (1). Breast cancer is the most common cancer among women, and according to statistics from the Iranian Ministry of Health, one in 15 women is at risk of developing the disease. In addition, the age of onset of breast cancer in Iranian women is at least a decade younger than in developed countries (2). It is a highly heterogeneous disease and the second leading cause of cancer death. The interaction of hereditary and environmental risk factors leads to the progressive accumulation of epigenetic and genetic changes in breast cancer cells (3). Though epidemiological evidence suggests specific risk factors such as old age, obesity, alcohol, and estrogen use, a family history of breast cancer is the strongest risk factor for the disease (2).

Although the exact genetic mechanisms responsible for most familial breast cancers have not yet been discovered, studies have shown that about half of these cancers are caused by mutations in the reproductive lineage of tumor suppressor genes and breast cancer cell signaling pathways (4). Studies showed that about 10% of human breast cancers are associated with hereditary genetic cancers such as deletions and polymorphisms. Growth factor receptors play an essential role in initiating the signaling pathways of cell proliferation and survival in the breast cells and other epithelial tissues. These receptors have an extracellular ligand-binding region, an intermembrane region, and a cytoplasmic domain-containing tyrosine kinase that can activate the downstream signal cascade (5). There is ample evidence that ERBB family growth receptors like epidermal growth factor receptor (EGFR), ERBB2, ERBB3, and ERBB4 are involved in the mechanism of the development of breast cancer and could provide potential targets for the treatment of the disease (6). Erb-B2 receptor tyrosine kinase 4 (ERbB4) is one of the four members of

the EGFR subfamily gene and is located on human chromosome 2 (2q34). The gene encodes receptor tyrosine-protein kinase erbB-4, a single-pass type I transmembrane enzyme that is a member of the epidermal growth factor receptor family (7). Previous surveys showed that due to their significant cellular functions in growth regulation, apoptosis, and cell proliferation, genetic variants of this gene are associated with cancer, congestive heart failure, and schizophrenia susceptibility in European and Asian populations (8, 9). Genome-wide association studies (GWAS) showed that ERbB4 rs13393577 polymorphism is associated with the risk of breast neoplasm in Korean people. In addition, rs7564590, rs905883, and rs7558615 have been linked to the increased risk of breast cancer, while rs1595066 has been revealed to decrease the risk of breast cancer (8, 10, 11).

2. Objectives

ERbB4 rs13393577 polymorphism had been related to many neoplasms as previously reported (12-14), furthermore, there is no evidence about this polymorphism associations with breast cancer in Iranian population while other SNPs of this gene is widely studied. The present study aimed to evaluate the impact of ERbB4 rs13393577 A>G polymorphism on the risk of developing breast cancer in the Iranian population.

3. Methods

3.1. Sampling

This case-control study was conducted on 110 patients with histopathologically confirmed breast cancer undergoing chemotherapy and 110 age and gender-matched healthy women. Healthy participants were unrelated with no history of any cancer. The current study is approved by the medical ethics committee of Islamic Azad University. Subjects blood samples were collected by venipuncture technique from individuals with written informed consent referred to Tehran general hospitals, Iran. The tubes code-named to observe the anonymity of participants. The population composition of the metropolis of Tehran represents a proper example for investigations about the Iranian public. Patients with incomplete data and without written informed consent were excluded from the study. Women with a hysterectomy and artificial menopause or those who were exposed to any type of radiation or chemotherapy during their lifetime were excluded from the study. The control group included women without a history of breast cancer or any other malignant disease, as well as none of their relatives with a history of breast cancer.

3.2. DNA Extraction

Five milliliters of blood specimens with EDTA anticoagulants were drawn from all participants to isolate genomic DNA. As previously described (15), DNA from blood samples was later lysed with sodium dodecyl sulfate to obtain leukocytes and proteinase K treatment of buffy coat. Phenol–chloroform and ethanol (Merck, Germany) precipitation were both used to purify the DNA. The extracted DNA was stored at 4°C. Spectrophotometric analysis (Nanodrop, Eppendorf, Germany) and agarose gel electrophoresis were used to check the quality and the quantity of the extracted DNA.

3.3. Tetra ARMS PCR

Genotyping of ERBB4 rs13393577 A>G polymorphism determined using the tetra-primer amplification refractory mutation system-polymerase chain (ARMS-PCR) reaction. For primer designing, the sequence surrounding the ERBB4 gene was first taken referring to NCBI online gene bank. Then, primers were designed using the Pick Primer tool, and final analyses were done via Oligo7 software (Table 1).

Each Tetra ARMS PCR reaction was performed in a final volume of 25 μL including 0.25 μL of 5 u/μL Taq DNA polymerase (Sigma Aldrich, Germany),1.5 μL of 50mM of $MgCl_2$, 100 ng of participants DNA, 1.5 μL of a 10 μM of four mixed dNTPs, 3.5 μL of 10x solution buffer and 10 pmol primers. A temperature gradient and $MgCl_2$ concentration gradient were performed to obtain optimum time and temperature for reaction. Finally, the PCR reaction was set as follows: (1) initial denaturation in 95°C for 3 minutes; (2) 30 cycles in 95°C for 30 seconds; (3) annealing in 50°C for 30 seconds; (4) initial replication in 70°C for 60 seconds; and (5) final extension in 71°C for 8 minutes. The tetra ARMS PCR reaction products were analyzed using 2% agarose gel (containing 0.5 μg/mL ethidium bromide) electrophoresis alongside a 1000 bp ladder. Afterward, PCR product sequences were analyzed using an automated DNA sequencer based on the Sangar method.

3.4. Statistical Analysis

Statistical analysis was performed using SPSS ver.19 software using *t*-test. The relationship between the presence of this polymorphism and disease and prognosis was calculated by computing odds ratio (OR) and 95% confidence intervals (CI) from logistic regression analysis. A P-value < 0.05 was considered statistically significant.

Table 1. List of Primer Sequences and Length of the Amplified Segment for Genotyping of Rs13393577 A>G Polymorphism

Primer Name	Sequence	Size
T allele	GTTGTCTGTTCTTTGGTCTATCGCT (5'- 3'):	199 bp
C allele	CATCCCTCAAGGTGATAGCACCG (5'- 3'):	275 bp
Outer primer		426 bp
Forward (5'- 3'):	CCCAACATTCATTGTCCTCTTTCC	
Reverse (5'- 3'):	TTACACTCTGGAAGAAAAGGCATTTACA	

4. Results

4.1. Study Population

In this study, 108 women and two men with breast cancer were examined by three experts, and mammography, sonography, and the genetic test confirmed breast cancer were recruited as a group of patients. 108 healthy women and two healthy men with no history of cancer or systematic diseases were included in the study as a control group. The mean age of patients was 44 $\pm$ 1.27 years and the mean age of controls was 45 $\pm$ 3.4 years.

4.2. Molecular Analysis Data

ARMS reaction used to screen ERBB4 rs13393577 T>C polymorphism (Figure 1). The results of the PCR study and sequencing (Figure 2) in the patient group showed that except for 17 cases that were heterozygous for T and C alleles, all cases were homozygous. The results of the statistical analysis summarized in Table 2. Figure 2 is showing the results of sequencing in patients with heterozygous alleles.

Allelic frequency in patients and control group for T and C alleles were 95, 97, 5, and 3% respectively. There was no statistically significant difference between C and T allele frequency between groups. The results of statistical analysis presented that the study population is in Hardy-Weinberg equilibrium and there is no difference between the patient's group and the control group.

5. Discussion

More than 1.3 million people are diagnosed with breast cancer each year, and about half a million die from the disease (16). Although several studies have been performed on the EGFR family gene in healthy and cancerous breast tissues, the association between some of its polymorphisms and the prognosis of the disease remains unclear (17, 18). In the current study, we have developed a tetra ARMS PCR method for the detection and genotyping of ERBB4 rs13393577 T>C polymorphism. Our data showed there was no significant correlation between this single nucleotide polymorphism and breast cancer. Hashemi et al. evaluated the relationship between the ERBB4 gene and the risk of prostate cancer and showed that there was no significant association between ERBB4 polymorphisms and the risk of cancer. They report 90, 16, and zero percent of frequency for AA, AG, and GG genotypes in patients respectively (19). These data are in line with our findings about the frequency of these genotypes of ERBB4 gene in the Iranian population.

A genome-wide association study by Kim et al. (2012) showed that the ERBB4 rs13393577 variant is associated with breast cancer susceptibility in Korean women (8). Mansouri Bidkani et al. evaluated the ErbB4 receptor polymorphism 2368A>C and risk of breast cancer and showed that rs13423759 allele C is significantly associated with the enhanced risk of breast cancer, elevated metastasis, and HER2 positivity (12). Similarly, Rokavec et al. (13), showed that the rs62626348 variant of the ERBB4 is related to breast and colon cancer but they report that other variants of this gene were not associated with cancer susceptibility. Our study is in line with those studies that do not link rs13393577 polymorphism to the risk of breast cancer. This discrepancy in the data can be attributed to the investigation design, environmental background, and population genetic composition. It must be pointed out that there were some limitations on the current survey.

5.1. Conclusion

This study assessed the association of ERBB4 rs13393577 polymorphism on breast cancer susceptibility in the Iranian population. Our data revealed that there is no relation between ERBB4 rs13393577 T>C polymorphism and breast cancer.

Footnotes

Authors' Contribution: Study concept and design, acquisition of data, analysis and interpretation of data and drafting of the manuscript, Rozita Eetezadi; Golnaz Asaadi Tehrani, administrative, technical, and material support, Kumarss Amini.

Conflict of Interests: None.

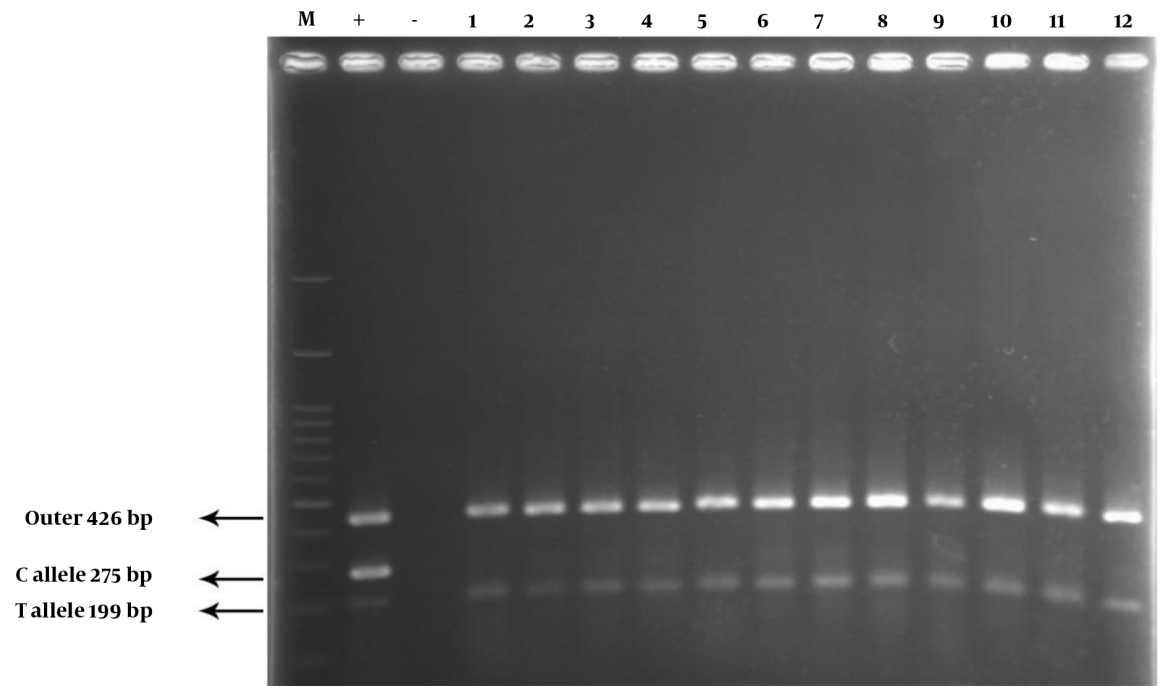

Figure 1. Electrophoresis of PCR product of patients' samples using specific primers. T allele (199bp), C allele (275 bp), and Outer primer (426 bp) (M, marker; +, positive control; -, negative control).

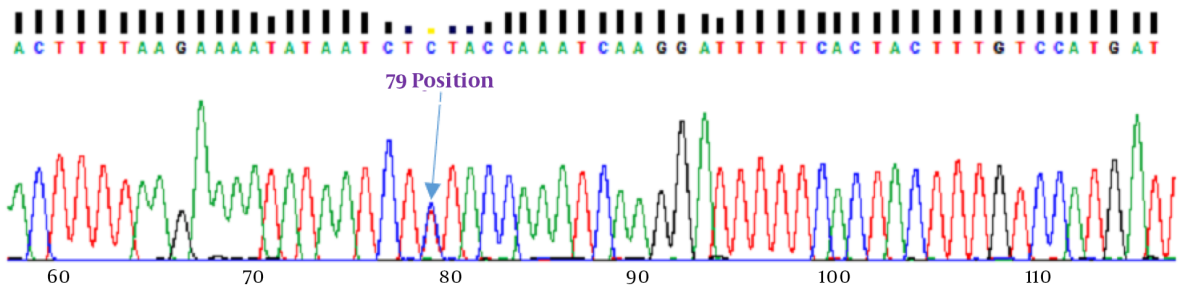

Figure 2. The results of sequencing in patients with heterozygous alleles show C to T polymorphism at position 79

Table 2. Genotype and Allele Frequencies of ERBB4 Polymorphism in Participants

rs13393577 A>G	Patients (%)	Controls (%)	Odd Ratio (95% CI)	P Value
TT	81 (73.63)	95 (86.36)	1.04 (0.02 - 53.58)	-
TC	17 (15.45)	13 (11.81)	0.32 (0.03 - 3.18)	0.031
CC	1 (0.90)	2 (1.81)	0.96 (0.01 - 49.34)	0.989

Ethical Approval: The current study was approved by the medical ethics committee of Islamic Azad University.

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019;**69**(1):7–34. doi: 10.3322/caac.21551. [PubMed: 30620402].
2. Mohammadi E, Aminorroaya A, Fattahi N, Azadnajafabad S, Rezaei N, Farzi Y, et al. Epidemiologic pattern of cancers in Iran; current knowledge and future perspective. *J Diabetes Metab Disord*. 2021;**20**(1):825–9. doi: 10.1007/s40200-020-00654-6. [PubMed: 34222092]. [PubMed Central: PMC8212225].
3. Britt KL, Cuzick J, Phillips KA. Key steps for effective breast cancer prevention. *Nat Rev Cancer*. 2020;**20**(8):417–36. doi: 10.1038/s41568-020-0266-x. [PubMed: 32528185].
4. Abril YLN, Fernandez IR, Hong JY, Chiang YL, Kutateladze DA, Zhao Q, et al. Pharmacological and genetic perturbation establish SIRT5 as a promising target in breast cancer. *Oncogene*. 2021;**40**(9):1644–58. doi: 10.1038/s41388-020-01637-w. [PubMed: 33479498]. [PubMed Central: PMC7935767].
5. Sporikova Z, Koudelakova V, Trojanec R, Hajduch M. Genetic markers in triple-negative breast cancer. *Clin Breast Cancer*. 2018;**18**(5):e841–50. doi: 10.1016/j.clbc.2018.07.023. [PubMed: 30146351].
6. Wang Z. ErbB receptors and cancer. *Methods Mol Biol*. 2017;**1652**:3–35. doi: 10.1007/978-1-4939-7219-7_1. [PubMed: 28791631].
7. Elster N, Toomey S, Fan Y, Cremona M, Morgan C, Weiner Gorzel K, et al. Frequency, impact and a preclinical study of novel ERBB gene family mutations in HER2-positive breast cancer. *Ther Adv Med Oncol*. 2018;**10**:1758835918778300. doi: 10.1177/1758835918778297. [PubMed: 30023006]. [PubMed Central: PMC6047239].
8. Kim HC, Lee JY, Sung H, Choi JY, Park SK, Lee KM, et al. A genome-wide association study identifies a breast cancer risk variant in ERBB4 at 2q34: Results from the Seoul Breast Cancer Study. *Breast Cancer Res*. 2012;**14**(2):R56. doi: 10.1186/bcr3158. [PubMed: 22452962]. [PubMed Central: PMC3446390].
9. Wang W, Zhao HF, Yao TF, Gong H. Advanced development of ErbB family-targeted therapies in osteosarcoma treatment. *Invest New Drugs*. 2019;**37**(1):175–83. doi: 10.1007/s10637-018-0684-8. [PubMed: 30353245].
10. Murabito JM, Rosenberg CL, Finger D, Kreger BE, Levy D, Splansky GL, et al. A genome-wide association study of breast and prostate cancer in the NHLBI's Framingham Heart Study. *BMC Med Genet*. 2007;**8 Suppl 1**. S6. doi: 10.1186/1471-2350-8-S1-S6. [PubMed: 17903305]. [PubMed Central: PMC1995609].
11. Zhu XL, Song FJ, Zheng H, Zhang LN, Zhao YR, Chen KX. Relationship between polymorphism of ErbB4 gene in mirco-RNA binding site and the risk for breast cancer. *Tumor*. 2011;**31**(3):233–8.
12. Mansouri Bidkani M, Tabatabaeian H, Parsafar S, Ghanei N, Fazilati M, Ghaedi K. ErbB4 receptor polymorphism 2368A>C and risk of breast cancer. *Breast*. 2018;**42**:157–63. doi: 10.1016/j.breast.2018.10.002. [PubMed: 30336339].
13. Rokavec M, Justenhoven C, Schroth W, Istrate MA, Haas S, Fischer HP, et al. A novel polymorphism in the promoter region of ERBB4 is associated with breast and colorectal cancer risk. *Clin Cancer Res*. 2007;**13**(24):7506–14. doi: 10.1158/1078-0432.CCR-07-0457. [PubMed: 18094435].
14. Segers VFM, Dugaucquier L, Feyen E, Shakeri H, De Keulenaer GW. The role of ErbB4 in cancer. *Cell Oncol*. 2020;**43**(3):335–52. doi: 10.1007/s13402-020-00499-4. [PubMed: 32219702].
15. Bigdeli R, Younesi MR, Panahnejad E, Asgary V, Heidarzadeh S, Mazaheri H, et al. Association between thrombophilia gene polymorphisms and recurrent pregnancy loss risk in the Iranian population. *Syst Biol Reprod Med*. 2018;**64**(4):274–82. doi: 10.1080/19396368.2018.1456576. [PubMed: 29658346].
16. Ginsburg O, Yip CH, Brooks A, Cabanes A, Caleffi M, Dunstan Yataco JA, et al. Breast cancer early detection: A phased approach to implementation. *Cancer*. 2020;**126 Suppl 10**:2379–93. doi: 10.1002/cncr.32887. [PubMed: 32348566]. [PubMed Central: PMC7237065].
17. Lev S. Targeted therapy and drug resistance in triple-negative breast cancer: The EGFR axis. *Biochem Soc Trans*. 2020;**48**(2):657–65. doi: 10.1042/BST20191055. [PubMed: 32311020].
18. Lin CH, Yang PJ, Lin SH, Yeh KT, Tsao TC, Chen YE, et al. Association between EGFR gene mutation and antioxidant gene polymorphism of non-small-cell lung cancer. *Diagnostics*. 2020;**10**(9). doi: 10.3390/diagnostics10090692. [PubMed: 32937815]. [PubMed Central: PMC7555708].
19. Hashemi M, Moradi N, Rezaei M, Sanaei S, Ziaee SAM, Narouie B, et al. ERBB4 gene polymorphisms and the risk of prostate cancer in a sample of Iranian Population. *Cell Mol Biol*. 2016;**62**(10):43–8. [PubMed: 27609473].