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Association between estrogen receptor alpha and aryl hydrocarbon receptor gene polymorphisms in the prognosis of breast cancer in Egypt

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Abstract

Breast cancer is the most malignant tumor among women in the world. Single nucleotide polymorphisms (SNPs) might better predict breast cancer prognosis. PvuII (T/C substitution), XbaI (A/G substitution), and aryl hydrocarbon (AhR) (G/A substitution) were evaluated as possible genetic prognostic factors for breast cancer. The aim of the current study was to assess the relation between PvuII (rs2234693), XbaI (rs9340799), and aryl hydrocarbon receptor gene polymorphisms AhR (rs2066853) in breast cancer prognosis. This was a case-control study that included 120 breast cancer patients classified into two groups. The first group included 60 patients with good prognostic factors, and the second group included 60 patients with poor prognostic factors. Blood samples were taken from all study participants to perform the genotyping assay. We found that positive genotypes of PvuII, XbaI, and AhR polymorphisms were strongly associated with better prognostic factors for breast cancer patients, while negative genotypes of PvuII and XbaI were more and significantly prevalent in poor prognostic breast cancer patients. We conclude that PvuII T/C, XbaI G/A, and AhR G/A alleles may be prognostic for breast cancer progression.

Keywords: breast cancer, aryl hydrocarbon receptor, XbaI, PvuII and prognostic factors

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Introduction

In Egypt, breast cancer is the most common malignancy in women, with the estimated number of patients being about 22,700 in 2020 and forecasted to be approximately 46,000 in 2050.[1] Early detection of molecular abnormalities in breast cancer may be an imperative strategy for assessment of prognosis, and treatment selection. According to the heterogeneity of this disease, management and prognosis are contingent on several prognostic features. However, even patients with similar prognostic features may have different clinical outcomes.[2] Tumoral genetic profiling has added prognostic

information to traditional classifications also; several genetic alterations affect cancer susceptibility and may have predictive and prognostic value in cancer.[3] Single-nucleotide estrogen receptor (ESR1) gene polymorphisms reported for PvuII (*Proteus vulgaris*) rs2234693 and XbaI (*Xanthomonas poorrii*) rs9340799 might be associated with the development, progression, metastasis, and prognosis of breast cancer.[4] The aryl hydrocarbon receptor (AhR) ligands may influence tumorigenic outcomes, particularly in aggressive breast tumors.[5] Steroid hormone receptors- estrogen receptor (ER) and progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and KI-67 (KI-67)[6] were investigated as prognostic markers. This study aimed to assess the relation between PvuII (rs2234693), XbaI (rs9340799), and aryl hydrocarbon receptor gene polymorphisms AhR (rs2066853) in breast cancer prognosis.

Patients and Methods

This case-control study was carried out at the Department of Oncology, Department of Clinical Pathology, and Suez Canal University Hospitals in Ismailia during the period from February 2020 to January 2022.

The data on breast cancer patients was obtained from their medical records after they underwent routine clinical and pathological investigations. Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2, and the cell proliferation marker (KI-67) were collected from the archives of the Department of Pathology, Suez Canal University Hospitals in Ismailia.

Study subjects were females aged 30 to 70 years diagnosed with breast cancer and confirmed by surgical biopsies and mammography. We excluded females with any other chronic disease except breast cancer. Study subjects were categorized into two groups: a group with good prognostic factors (estrogen receptor-positive, progesterone-positive, HER2-negative, less than 14% antigen KI-67 index) and the second group with poor prognostic factors for breast cancer (estrogen receptor–negative, progesterone-negative, HER2-positive, ≥ 14% KI-67 index) according to classification of the World Health Organization[7]. ER and PR were positive when ≥1% of the tumor cells showed positive nuclear staining. HER2 was scored negative if no staining or membrane staining in less than 10% of invasive tumor cells was seen. HER2 was positive if weak, moderate, or strong complete membrane staining was seen by immunohistochemistry.

A blood sample (about 2 ml) was collected from each study participant. Genomic DNA was extracted from whole blood samples by using commercial DNA extraction Kits (Catalog no. 69504, DNeasy® Blood & Tissue Kit, QIAGEN, Germany), according to the manufacturer's instructions. Extracted DNA samples were stored at -20°C until used. SNPs were genotyped by using specific primers and probes from (Applied Biosystem, Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's instructions. XbaI SNP primers were forward: 5'-CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3', reverse: 5'-TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3', sequence [VIC/FAM]: TTCCCAGAGACCCTGAGTGTGGTCT[A/G]GAGTTGGGATGAGCATTGGTCTCTA.[5] PvuII SNP primers were forward: 5'CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3', reverse: 5'TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3', sequence [VIC/FAM]: TCATCTGAGTTCCAAATGTCCCAGC[C/T]GTTTTATGCTTTGTCTCTGTTTCCC.[8] AhR SNP primers, forward 5'-GATTGATTTTGAAGACCTCA-3', reverse 5'-CTGAAGGTATGAAGGGAG-3', sequence [VIC/FAM]: CTAGGCATTGATTTTGAAGACATCA[A/G]ACACATGCAGAATGAAAAATTTTTC[8, 9]

The PCR reaction mix volume was 10 µl TaqMan Universal PCR Master Mix and 1 µl primer mix were added into each well of a reaction plate. For ease of use, assay mixes were diluted to 20X working solutions with 1X TE buffer (10mM Tris-HCl, 1 mM EDTA, pH 8.0) and DNase-free water were used. A 5 µl of sample or control DNA were added for each reaction into the appropriate wells containing 40 ng of genomic DNA.

The reaction plates were loaded into the thermal cycler, incubated at 50°C for 02:00 minutes, polymerase activation at 95°C for

10:00 minutes, followed by denaturation at 95°C for 15 seconds, annealing/extending at 60°C for 01:00 minute. PCR denaturation and annealing were repeated for 40 cycles in a thermocycler (Corbett Research RG-6000 Rotor-Gene 5Plex HRM Real Time PCR & Rotor, UK). The plates were read by fluorescence measurements software system to determine which alleles were in each sample for later genotyping analysis. The system software recorded the results of the genotyping run on a scatter plot of allele 1 (VIC® dye) versus allele 2 (FAM™ dye).

Statistical Analysis

Data were statistically analyzed by using Statistical Package for Social Sciences (SPSS) software package version 22.0. Two types of statistics were done. Descriptive statistics included number (no) and percent (%) for qualitative data while standard deviation (SD) and mean for quantitative data. Analytic statistics; Chi-square test: used to compare between two or more groups. Statistically significant was set at *p*-value < 0.05.

Results

There were statistically significant differences between AhR allelic variants, progesterone receptors, and HER2 (*p*=0.03 and *p*=0.001, respectively). There were no statistically significant differences between AhR allelic variants, estrogen receptors, and KI-67 (*p*=0.1 and *p*=0.3, respectively) (Table 1).

Table 1. Distribution of clinicopathological prognostic factors according to AhR frequencies.

Prognostic factors	AhR (n, %)			*p*-value
	WT/WT	WT/A	A/A	
Hormonal status/Estrogen				
Negative	0 (0%)	1 (3.6%)	8 (10.8%)	NS
Positive	1 8 (100%)	27 (96.4%)	66 (89.2%)	
Hormonal status/Progesterone				
Negative	0 (0%)	4 (14.3%)	19 (25.7%)	0.03
Positive	18 (100%)	24 (85.7%)	55 (74.3%)	
Hormonal status/HER2 neu				
Negative	18 (27.7%)	17 (26.2%)	30 (46.2%)	0.001
Positive	0 (0%)	11 (20%)	44 (80%)	
KI-67				
Low	16 (88.9%)	21 (75%)	54 (73%)	NS
High	2 (11.1%)	7 (25%)	20 (27%)	

Chi-square test, $p > 0.05$ is not significant (NS), A = mutant AhR allele, WT = wild-type allele (normal allele)

There were statistically significant differences between XbaI allelic variants for the study groups; progesterone receptor, HER2 receptor, and KI-67 (*p*=0.003, *p*=0.001and *p*=0.01, respectively). No difference was observed between XbaI allelic variants and estrogen receptors (*p*=0.2), (Table 2).

Table 2. Distribution of clinicopathological prognostic factors according to XbaI frequencies.

Prognostic factors	XbaI (n, %)			*p*-value
	WT/WT	WT/G	G/G	
Hormonal status /Estrogen				
Negative	2 (5.3%)	2 (4.3%)	5(13.9%)	NS
Positive	36 (94.7%)	44 (95.7%)	31(86.1%)	
Hormonal status /Progesterone				
Negative	2 (5.3%)	8 (17.4.%)	13 (36.1%)	0.003
Positive	36 (94.7%)	38(82.6%)	23 (63.9%)	
Hormonal status /HER2 neu				
Negative	31 (47.7%)	27 (41.5%)	7 (10.8%)	0.001
Positive	7 (12.7%)	19 (34.5%)	29 (52.7%)	
KI-67				
Low	34 (89.5%)	35 (76.1%)	22 (61.1%)	0.01
High	4 (10.5%)	11(23.9%)	14 (38.9%)	

Chi-square test, $p > 0.05$ is not significant (NS), G = mutant XbaI allele, WT= wild-type allele (normal allele).

There were statistically significant differences between PvuII allelic variants for the study groups; estrogen status, progesterone, HER2 and KI-67 (p=0.05, p=0.01, p=0.001 and p=0.03, respectively) (Table 3).

Table 3. Distribution of clinicopathological prognostic factors according to PvuII frequencies.

Prognostic factors	PvuII (n, %)			*p*-value
	WT/WT	WT/C	C/C	
Hormonal status /Estrogen				
Negative	0 (0%)	4 (44.4%)	5 (13.2%)	0.05
Positive	29 (100%)	49 (92.5%)	33 (86.8%)	
Hormonal status /Progesterone				
Negative	2 (6.9%)	5 (9.4%)	16 (42.1%)	0.01
Positive	27 (93.1%)	48 (90.6%)	22 (57.9%)	
Hormonal status /HER2 neu				
Negative	25 (38.5%)	31 (47.7%)	9 (13.8%)	0.001
Positive	4 (7.3%)	22 (40%)	29 (52.7%)	
KI-67				
Low	25 (86.2%)	43 (81.1%)	23 (60.5%)	0.03
High	4 (13.8%)	10 (18.9%)	15 (39.5%)	

Chi-square test, *p*-value is significant at <0.05, G = mutant XbaI allele, WT= wild-type allele (normal allele).

Discussion

Breast cancer develops silently, while mortality is declining due to improvements in screening programs and treatment. Several genetic factors affect cancer susceptibility and may have a prognostic value in cancer. Our study intended to assess the relation between PvuII (rs2234693), XbaI (rs9340799), and AhR (rs2066853) gene polymorphisms in the prognosis of breast cancer.

In agreement with Long et al., 2006 suggestion, we reported a higher distribution of mutant genotypes AA and heterogeneous genotypes GA of AhR rs2066853 polymorphism in positive estrogen subjects than wild-type GG without a significant association between AhR allelic variants and estrogen status.[10] Contrasting our proposition, Tryggvadottir et al., 2021, suggested that AhR was strongly associated with patients' ER-negative.[11] However, similar to Tryggvadottir et al., 2021 study findings, we reported that there was a statistically significant difference between AhR allelic variants and progesterone status.[11] However, this was not in agreement with findings of a study by Vacher et al., 2018.[12] Benoit et al., 2022 suggested that there was over-expression of AhR in HER2 negative cells but not over-expression in HER2 receptor.[5]

In the present study, we found a statistically significant difference between AhR allelic variants and HER2 in breast cancer patients. Vacher et al., 2018 suggested that AhR expression was not implicated in breast cell proliferative activity. In the present study, we revealed no statistically significant difference between AhR allelic variants and KI-67 in breast cancer patients.[12] Vogel et al., 2021 and Benoit et al., 2022 noted that AhR signaling in mammary fibroblasts and overexpression of the AhR were correlated to breast cancer progression.[5, 13]

Our work revealed a statistically significant difference between XbaI allelic frequencies and progesterone hormonal status, HER2, and the KI-67 index. Carrillo-Moreno et al., 2019 detected an association between the ESR1 XbaI (rs9340799) GG allele and progressive markers of breast cancer.[14] This is in contrast to Al-Eitan et al., 2019 hypothesis who reported that no significant association with breast cancer was found for the XbaI rs9340799 in Jordanian Arabs.[15] We reported no statistically significant difference between XbaI allelic variants and estrogen hormonal status. Our work revealed that PvuII allelic variants were statistically associated with estrogen hormonal status, progesterone, HER2, and KI-67. Our study revealed that breast patients who carried AhR (GG), XbaI (AA), and PvuII (TT) genotypes had a better prognosis, this agreed with findings of previous studies.[4, 11, 16] In conclusion, the polymorphic variation of positive genotypes of PvuII, XbaI, and AhR polymorphisms is highly associated with better prognosis in breast cancer patients. Negative genotypes of PvuII, XbaI, and AhR polymorphisms are strong and significantly prevalent in poor-prognostic breast cancer patients

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Author Contributions

SAA; designed, conducted the study, read and approved the final manuscript. EHME read and approved the final manuscript. FAMM designed, performed the analysis of collected data and wrote the manuscript. MAS collected data, read and approved the final manuscript.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee, Faculty of Medicine, Suez Canal University (reference number: Research 3971#; dated 28 October 2019).

Informed consent

Permission and informed consent were obtained from all participants before taking any data or doing any investigations.

References

1. Abd EL-Sabour Mohamed W, Abdallah Abdel-Mordy M, Said Sabry S. (2022).Health Belief Model among Post-Menopausal Women regarding Breast Cancer. *Journal of Nursing Science Benha University.* 3(2):338-355.

2. Susini T, Biglia N, Bounous VE. (2022).Prognostic Factors Research in Breast Cancer Patients: New Paths. Vol 14: MDPI; 971.

3. Leitão M, Lopes S, Pereira D, et al. (2022). Genetic Polymorphisms as Predictors of Survival in Breast Cancer: Future Lessons in Historical Data. *Cureus.* 14(1).

4. Sunemail T, Lian R, Liang X, et al. (2022). Association Between ESR1 XBAI and Breast Cancer Susceptibility: A Systematic Review and Meta-Analysis. *Clinical and Investigative Medicine.* 45(1):E21-34.

5. Benoit L, Jornod F, Zgheib E, et al. (2022). Adverse outcome pathway from activation of the AhR to breast cancer-related death. *Environment International.* 107323.

6. Courtney D, Davey MG, Moloney BM, et al. (2022). Breast cancer recurrence: Factors impacting occurrence and survival. *Irish Journal of Medical Science (1971-).* 191(6):2501-2510.

7. KIm J, KIm JY, Lee H-B, et al. (2020). Characteristics and prognosis of 17 special histologic subtypes of invasive breast cancers according to World Health Organization classification: comparative analysis to invasive carcinoma of no special type. *Breast Cancer Research and Treatment.* 184:527-542.

8. Sierra-Martinez M, HERnández-Cadena L, García-Sánchez JR, et al. (2018). Predictive polymorphisms for breast cancer in postmenopausal Mexican women. *Journal of Cancer Research and THERapeutics.* 14(3):640-646.

9. Huang S, Shui X, He Y, et al. (2015). AhR expression and polymorphisms are associated with risk of coronary arterial disease in Chinese population. *Scientific reports.* 2015;5(1):1-7.

10. Long J-R, Egan KM, Dunning L, et al. (2006). Population-based case–control study of AhR (aryl hydrocarbon receptor) and CYP1A2 polymorphisms and breast cancer risk. *Pharmacogenetics and genomics.* 16(4):237-243.

11. Tryggvadottir H, Sandén E, Björner S, et al. (2021). The Prognostic Impact of Intratumoral Aryl Hydrocarbon Receptor in Primary Breast Cancer Depends on the Type of Endocrine THERapy: A Population-Based Cohort Study. *Frontiers in Oncology.* 11:642768.

12. Vacher S, Castagnet P, Chemlali W, et al. (2018). High AHR expression in breast tumors correlates with expression of genes from several signaling pathways namely inflammation and endogenous tryptophan metabolism. *PLoS One.* 2018;13(1):e0190619.

13. Vogel CF, Lazennec G, Kado SY, et al. (2021). Targeting the aryl hydrocarbon receptor signaling pathway in breast cancer development. *Frontiers in immunology.* 12:625346.

14. Carrillo-Moreno DI, Figuera LE, González GZ, et al. (2019). Association of rs2234693 and rs9340799 polymorphisms of ESR1 gene in breast cancer of Mexican population. *J BUON.* 2019;24(5):1927-1933.

15. Al-Eitan LN, Rababa'h DM, Alghamdi MA, et al. (2019). Association of CYP gene polymorphisms with breast cancer risk and prognostic factors in the Jordanian population. *BMC medical genetics.* 20(1):1-12.

16. Karsono R, Haryono SJ, Karsono B, et al. (2021). ESR1 PvuII polymorphism: from risk factor to prognostic and predictive factor of the success of primary systemic tHERapy in advanced breast cancer. *BMC cancer.* 21(1):1-7.