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Evaluation of PvuII (rs2234693) and AhR (rs2066853) Gene Polymorphisms as Prognostic Markers in Breast Cancer

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Abstract

Background: **the most common cancer detected in females is breast cancer. Several genetic alterations may have prognostic effect on breast cancer. Single-nucleotide polymorphisms might predict breast cancer management. Single nucleotide polymorphisms of PvuII (T/C substitution) and aryl hydrocarbon (G/A substitution) were estimated as probable genetic prognostic factors for breast cancer.** ***Aim:*** **to assess the relation between estrogen receptor alpha PvuII (rs2234693) and aryl hydrocarbon receptor gene polymorphisms (rs2066853) in breast cancer prognosis.** ***Material and method:*** **this study is case-control that enrolled 120 breast cancer patients categorized into two groups: the first one involved 60 females with good prognostic factors, the second group included 60 females with poor prognostic factors. Genotyping assay were done by a real-time polymerase chain reaction.** ***Results:*** **our finding revealed that the allelic frequency of wild-type genotypes for PvuII and AhR polymorphisms was associated with patients who had better prognosis for breast cancer. Their mutant genotypes were significantly associated with poor prognoses in breast cancer patients. Conclusion: PvuII, AhR genotypes were statistically significant associated with breast cancer prognosis.**

Keywords: Genetic factors, Estrogen receptor, aryl hydrocarbon, prognosis.

Introduction

Management of breast cancer is greatly individualized and depends on prognostic factors(1). Breast cancer molecular abnormalities play an imperative role in early detection, prognosis valuation, and compatible treatment selection(2). Estrogen receptor-α and Estrogen receptor-β, are main regulators for the estrogens actions, ER-α gene enables to encode a

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transcription factor with an estrogen response element DNA-binding domain [3]. ERα gene is a steroid hormone receptor gene, located on chromosome 6 at 6p25.1 and ERα gene mutation is able to prompt cell proliferation, regulate cell apoptosis by affecting protein expression, and play a role in the development of breast cancer [4]. A single-nucleotide polymorphism (SNP) of the estrogen receptor1 gene (ESR1), PvuII (rs2234693) polymorphism, mapped to the first intron of 397 bp of exon 2 [5], may be related to breast cancer progression, and prognosis [6]. Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that regulates genes in response to exposure to environmental polyaromatic hydrocarbons, AhR emerging factors targeted for treating certain subtypes of metastatic breast cancer [7]. AhR is overexpressed and constitutively stimulated in advanced breast cancer cases and drives the progression of breast cancer [8, 9]. Our study aimed to determine the association between PvuII (rs2234693), and AhR (rs2066853) allelic variants and breast cancer prognostic factors.

Methods

A case-control study was conducted between February 2020 and January 2022 at the Department of Oncology, Clinical Pathology, and Suez Canal University Hospital in Ismailia. Due to the World Health Organization, patients diagnosed with breast cancer were divided into two groups according to the breast cancer prognostic factors such as; tumor stage, histologic grade, tumor size, regional lymph nodes, lymphovascular invasion, margin status, menopausal state, local/regional relapse and distant metastasis [10]. The first group included good prognosis breast cancer (BC) patients, and the second included poor prognosis BC patients.

Ethical approval

The study was approved by the Research Ethics Committee, Faculty of Medicine, Suez Canal University, (reference number: Research 3971#; dated 28 October 2019). The purpose and Protocols of the study were explained to patients, and written agreement was got from all the study subjects. The study was approved by the Research

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Inclusion criteria

Females whose diagnosis was histologically confirmed as breast cancer

Exclusion criteria

Females with any other chronic disease except breast cancer

Breast cancer patients who have incomplete or duplicate data

Data collection

A detailed history of each patient was obtained from their medical records.

Genetic preparation

Sampling of 2 mL peripheral venous blood samples were obtained in sterile tubes containing EDTA. Genomic DNA was extracted from the whole blood using DNeasy® Blood & Tissue Kit, by QIAGEN

(Catalog no. 69504) consistent with the manufacturer's instructions. DNA samples were preserved at -20°C until analysis. TaqMan® Universal PCR Master Mix (Applied Biosystems, CA, USA), specific primers and probes from Applied Biosystem, Thermo Fisher Scientific, Inc.,Waltham, MA,USA, and the specific sequence of PvuII SNP primers (rs2234693C/T) transition substitution, Assay ID: C_3163590_[10], location chr.6:151842200 on Build GRCh38, Forward 5'CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3'. Reverse: 5'TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3'[11], specific sequence of AhR SNP primers (rs2066853 A/G) transition substitution Assay ID: C_11170747_20, location chr.7:17339486 on Build GRCh38, Forward 5′-GATTGATTTTGAAGACCTCA-3′ Reverse 5′-CTGAAGGTATGAAGGGAG-3′ were used [12].

Principle of DNA extraction

The lysate buffering conditions were adjusted to allow optimal binding of the DNA to the DNeasy Mini spin column membrane before the sample was loaded onto the DNeasy Mini spin column. DNA was adsorbed onto the DNeasy Mini spin column silica membrane during brief centrifugation. Salt and pH conditions in the lysate ensure that protein and other contaminants, which can inhibit PCR (polymerase chain reaction) and other downstream enzymatic reactions, are not retained on the DNeasy Mini spin column membrane. DNA bouto the DNeasy Mini spin column membrane was washed by two centrifugations. The use of two different wash buffers, Buffer AW1 and Buffer AW2, significantly improves the purity of the eluted DNA. Wash conditions ensure the complete removal of any residual contaminants without affecting DNA binding. Reaction volume: 20 µl (Pipette the PCR reaction mix 10 µl of TaqMan Universal PCR Master Mix, 1 µl primer mix (sense and antisense at 36 µM each, and 8 µM of the dye-labelled probe) into each well of a reaction plate, Pipette 5 µL of sample or control DNA for each reaction into the appropriate wells containing 40 ng genomic DNA. The reaction conditions for PCR were 40 cycles at 95°C for 10 minutes, 92°C for 15 seconds, and 50°C for 2 minutes in a thermocycler (RCorbett research model RG6000).

Statistical Analysis

Microsoft Excel for Windows Office 2010 and statistically analyzed by Statistical Package for Social Sciences software package version 22.0, the Chi-square test, Kruskal-Wallis test, Fisher's exact test, the odds ratio, their 95% confidence interval, and the Kaplan-Meier method.

Result

This study enrolled 120 patients with breast cancer, divided into two groups due to the World Health Organization, 2019 [13]. Tumor size, stages, , regional lymph nodes, histologic grade, lymphatic vascular system invasion, margins, local/regional recurrence, and distant metastases were significantly associated with breast cancer prognosis with p-values of 0.005, 0.005, 0.005, 0.005, 0.001, 0.001, 0.001, 0.01, respectively. Most patients in both groups were post-menopausal and wasn't statistically significant

(p-value of 0.4), as shown in table 1. Stage group, tumor size, regional lymph nodes, histologic grade, margins, local or regional recurrence, and distant metastases of study subjects were statistically significant with pvull allelic variants, while lymphatic vascular system and menopausal state were not statistically significant with pvull allelic variants, as shown in table 2. Breast cancer stages, tumor sizes, regional lymph nodes, histologic grade, and local and regional recurrence were statistically significant with AhR allelic variants for the study population. Statistically significant differences between AhR allelic variants, pathological type, lymphatic vascular system, margins, menopausal state, and distant metastases weren't found, as shown in table 3.

In table 4, the prevalence of wild-type WT/WT for AhR and PvuII polymorphisms increased in good prognostic breast patients. Interestingly, the presence of the A/A for AhR and C/C for Pvull genotypes was higher in poor prognostic breast cancer patients with p-value = 0.001.

Discussion

Breast cancer accounts for more than 1 in 10 new cancer diagnoses each year [14]. Breast cancer develops silently although mortality is decreasing owing to improvements in prognosis and treatment. According to the heterogeneity of this disease, management and prognosis are contingent on several prognostic features. Patients with similar prognostic characters may have dissimilar clinical results [15]. Currently, tumoral genetic profiling has additional prognostic information also, several genetic factors may have predictive and prognostic effects on cancer [16]. We aimed to evaluate the relation between estrogen receptor alpha Pvull (rs2234693), and AhR (rs2066853) gene polymorphisms in breast cancer prognosis. This study was carried out on 120 patients who had previously been confirmed diagnosed with breast cancer and were attending Suez Canal University Hospital. According to Courtney Donald et al., study, we classified our study population into two groups: good and poor prognostic breast cancer patients according to several clinicopathological prognostic factors such as; stages, histologic grade, tumor size, numbers of axillary lymph nodes, vascular or lymphatic system invasion, margin, local or regional recurrence and distant metastases [17]. The study patients' prognostic factors, PvuII (T/C substitution) and AhR (G/A substitution) gene polymorphisms, were investigated for each study group. ERα single nucleotide polymorphism is sited in the first intron and is the c454-397T>C-site polymorphism [18]. This polymorphism is 397 base pairs upstream of exon 2 and is celebrated by restriction enzymes, PvuII (rs2234693) [19]. Al-Amri et al. suggested that PvuII (rs2234693) in the ESR1 gene was not related to menopausal women with breast cancer [20]. Our study revealed that there was no statistically significant difference between Pvull allelic variants and menopausal status in breast cancer patients.

Table 1: Distribution of patients' clinical characteristics in the study population

Prognostic values	First group	Second group	P value
Stage group			
IA	38 (63.3%)	0 (0%)	0.005*
IB	10 (16.7%)	0 (0%)	
IIA	10 (16.7%)	16 (26.7%)	
IIB	2 (3.3%)	13 (21.7%)	
IIIA	0 (0%)	9 (15%)	
IIIB	0 (0%)	7 (11.7%)	
IIIC	0 (0%)	8 (13.3%)	
IV	0 (0%)	7 (11.7%)	
Tumor size (T)			
T1	49 (81.7%)	5 (8.3%)	0.005*
T2	9 (15%)	31 (51.7%)	
T3	2 (3.3%)	13 (21.7%)	
T4	0 (0%)	11 (9.2)	
Regional lymph nodes (n)			
N0	37 (61.7%)	1 (1.7%)	0.005*
N1a	16 (26.7%)	19 (31.7%)	
N1b	7 (11.7%)	12 (20%)	
N2a	0 (0%)	12 (20%)	
N2b	0 (0%)	8 (13.3%)	
N3a	0 (0%)	5 (8.3%)	
N3b	0 (0%)	3 (5%)	
Histologic grade			
Grade I	23 (38.3%)	0 (0%)	0.005*
Grade II	37 (61.7%)	37 (61.7%)	
Grade III	0 (0%)	23 (38.3%)	
Lymphatic vascular system invasion			
Negative	60 (100%)	47 (78.3%)	0.001*
Positive	0 (0%)	13 (21.7%)	
Margins			
Negative	60 (100%)	50 (83.3%)	0.001*
Positive	0 (0%)	10 (16.7%)	
Menopausal state			
Pre-menopause	22 (36.7%)	25 (41.7%)	0.4
Post-menopause	38 (63.3%)	35 (58.3%)	
Local/Regional Recurrence			
Negative	50 (83.3%)	17 (28.3%)	0.001*
Positive	10 (16.7%)	37 (61.7%)	
Distant metastases (M)			
M0	60 (100%)	54(90%)	0.01*
M1	0 (0%)	6 (10%)	

Table 2: Distribution of patients' clinical characteristics due to pvull frequencies				
	Pvull (n,%)			**P-value**
Prognostic factors	**WT/WT**	**WT/C**	**C/C**	
Stage group				
IA	15 (51.7%)	18 (34%)	5 (13.2%)	0.004*
IB	4 (13.8%)	6 (11.3%)	0 (0%)	
IIA	6 (20.7%)	10 (18.9%)	10 (26.3%)	
IIB	2 (6.9%)	9 (17%)	4 (10.5%)	
IIIA	1 (3.4%)	3 (5.7%)	5 (13.2%)	
IIIB	1 (3.4%)	2 (3.8%)	4 (10.5%)	
IIIC	0 (0%)	4 (7.5%)	4 (10.5%)	
IV	0 (0%)	1 (1.9%)	6 (15.8%)	
Tumor size (T)				
T1	21(72.4%)	27 (50.9%)	6 (15.8%)	0.01*
T2	5 (17.2%)	17(32.1%)	18 (47.4%)	
T3	2 (6.9%)	7 (13.2%)	6 (15.8%)	
T4	1 (3.4%)	2 (3.8%)	8 (21.1%)	
Regional lymph nodes (n)				
N0	14 (48.3%)	18 (34%)	6 (15.8%)	0.02*
N1a	9 (31%)	19 (35.8%)	7 (18.4%)	
N1b	4 (13.8%)	6 (11.3%)	9 (23.7%)	
N2a	0 (0%)	4 (7.5%)	8 (21.1%)	
N2b	2 (6.9%)	2 (3.8%)	4 (10.5%)	
N3a	0 (0%)	3 (5.7%)	2 (5.3%)	
N3b	6 (15.8%)	1 (1.9%)	2 (5.3%)	
Histologic grade				
Grade I	10 (34.5%)	11 (20.8%)	2 (6.9%)	0.01*
Grade II	17 (58.6%)	37 (69.8%)	20 (52.6%)	
Grade III	2 (5.3%)	5 (9.4%)	16 (42.1%)	
Lymphatic vascular system				0.2
Negative	28 (96.6%)	48 (90.6%)	32(84.2%)	
Positive	1(3.4%)	5 (9.4%)	6(15.8%)	
Margins				
Negative	29 (100%)	48 (90.6%)	33(86.8%)	0.05*
Positive	0 (0%)	5 (9.4%)	5(13.2%)	
Menopausal state				
Pre-menopause	10 (34.5%)	19 (35.8%)	18 (47.4%)	0.5
Post-menopause	19 (65.5%)	34 (64.2%)	20 (52.6%)	
Local/Regional Recurrence				
Negative	22 (75.9%)	32 (60.4%)	13 (34.2%)	0.001*
Positive	7 (24.1%)	21 (39.6%)	19 (50%)	
Distant metastases (M)				
M0	29 (100%)	53 (100%)	32 (84.2%)	0.01*
M1	0 (0%)	0 (0%)	6 (5%)	

Table 3: Distribution of patients' clinical characteristics due to AhR frequencies

Prognostic factors	AhR (n, %)			P-value
	WT/WT	WT/A	A/A	
Stage group				
IA	12 (66.7%)	11(39.3%)	15 (20.3%)	0.002*
IB	2 (11.1%)	4 (14.3%)	4 (5.4%)	
IIA	3 (16.7%)	4 (14.3%)	19 (25.7%)	
IIB	1 (5.6%)	6 (21.4%)	8 (10.8%)	
IIIA	0 (0%)	0 (0%)	9 (12.2%)	
IIIB	0 (0%)	1 (3.6%)	6 (8.1%)	
IIIC	0 (0%)	1 (3.6%)	7 (9.5%)	
IV	0 (0%)	1 (3.6%)	6 (8.1%)	
Tumor size (T)				
T1	15 (83.3%)	15 (53.6%)	24 (32.4%)	0.01*
T2	11.1 (15%)	8 (28.6%)	30 (30.5%)	
T3	1 (5.6%)	3 (10.7%)	11 (14.9%)	
T4	0 (0%)	2 (7.1%)	9 (12.2%)	
Regional lymph nodes (n)				
N0	12 (66.7%)	10 (35.7%)	16 (21.6%)	0.03*
N1a	4 (22.2%)	9 (32.1%)	22 (29.7%)	
N1b	2 (11.1%)	5 (17.9%)	12 (16.2%)	
N2a	0 (0%)	2 (7.1%)	10 (13.5%)	
N2b	0 (0%)	1(3.6%)	7 (9.5%)	
N3a	0 (0%)	1 (3.6%)	4 (5.4%)	
N3b	0 (0%)	0 (0%)	3 (4.1%)	
Histologic grade				
Grade I	6 (33.3%)	5 (17.9%)	12 (16.2%)	0.04*
Grade II	12 (66.7%)	18 (64.3%)	44 (59.5%)	
Grade III	0 (0%)	5 (17.9%)	18 (24.3%)	
Lymphatic vascular system				
Negative	18 (100%)	25 (89.3%)	65(87.8%)	0.1
Positive	0 (0%)	3 (25%)	9(12.2%)	
Margins				
Negative	18 (100%)	26 (92.9%)	66 (89.2%)	0.2
Positive	0 (0%)	2 (7.1%)	8 (10.8%)	
Menopausal state				
Pre-menopause	6 (33.3%)	8 (28.6%)	33 (44.6%)	0.3
Post-menopause	12 (66.7%)	20 (71.4%)	41 (55.4%)	
Local/regional recurrence				
Negative	17 (94.4%)	20 (71.4%)	30 (40.5%)	0.001*
Positive	1 (5.6%)	7 (25%)	39 (52.7%)	
Distant metastases (M)				
M0	18 (100%)	27 (96.4%)	69 (93.2%)	0.3
M1	0 (0%)	1(3.6%)	5 (6.8%)	

A = mutant AhR allele, WT= wild-type allele (normal allele), Chi-square test, * P-value is significant when (<0.05)

Table 4: Distribution of AhR and PvuII allelic variants in study groups					
Genotypes	Study groups		Odd Ratio (OR*)	Confidence Interval (95% CI)*	P-value
	Good prognosis BC patients	Bad prognosis BC patients			
AhR					
WT/WT	18 (30%)	0 (0%)			
WT/A	17 (28.3%)	11 (18.3%)	4.2	1.93-7.72	0.001*
A/A	25(41.7%)	49 (81.7%)			
PvuII					
WT/WT	25 (41.7%)	4 (6.7%)			
WT/C	29 (48.3%)	24 (40%)	4.5	1.77-11.43	0.001*
C/C	6 (10%)	32 (53.3%)			

Chi-square test, P-value is significant when (<0.05)*
, A = mutant AhR allele, G = mutant XbaI allele, C = mutant PvuII allele, WT = wild-type allele (normal allele)

In contrast, Houtsma et al. and Johansson et al. showed that the T allele of PvuII (rs2234693) in the ESR1 gene is associated with enhanced overall survival in postmenopausal women [21, 22]. Al-Amri et al., Ahrar et al., and Oliva et al. indicated a corroborating linkage in patients with advanced breast cancer outcomes with C allele carriers that stimulate tumor growth [20, 23, 24]. Our work revealed that PvuII allelic variants were statistically associated with stage, tumor size, local and regional recurrence, and distant metastases. Conversely, Karsono et al study negated that hypothesis [25]. Moreover, we reported that the PvuII (rs2234693) homozygous wild-type had a high distribution of good prognostic breast cancer outcomes. Moreover, the PvuII (rs2234693) mutant homozygous CC was associated with poor prognostic outcomes than those with the WT/WT genotype. We postulated that PvuII (rs2234693) considered a prognostic feature for breast cancer prognosis in the convention of Scalco et al. [26]. On the contrary, Liu et al. submitted that PvuII (rs2234693) is not significantly associated with breast cancer [27]. Also, Karsono et al. demonstrated that PvuII TT allelic variants have a better prognosis than PvuII CC [25]. By studying the association between AhR SNP rs2066853 (c1661G > A, Arg554Lys) gene polymorphisms and the prognosis of breast cancer, we reported that AhR allelic variants weren't associated with the menopausal state. According to studies conducted by Tryggvadottir et al. AhR GG had a favourable prognosis compared to patients with AhR AA [28]. In contrast to our hypothesis, Long et al. reported that the AhR Arg554Lys polymorphism was significantly associated with reduced risk of breast cancer in premenopausal females [29]. Similar to the Martnez-Ramrez et al. study, our study revealed no statistical association between the expression of AhR allelic variants and distant metastases [30]. Vogel et al. and Benoit et al. propositions were dissimilar to our supposition; they noted that AhR signalling in mammary fibroblasts and

proliferation and over-expression of AhR were correlated to proliferation, metastasis, and breast cancer progress [6, 8]. Interestingly, our findings demonstrated a strong association of AhR (rs2066853) homozygous wild-type genotype GG with better prognostic outcomes compared to mutant homozygous genotype AA associated with worse prognosis breast cancer outcomes. These data suggest that AhR (rs2066853) has a prognostic power for breast cancer. This could be attributed to the suggestion that AhR has a role in the improvement of breast cancer cells owing to AhR having a potential effect on breast cancer progression as it is convoluted in a variety of cellular developments such as cell cycle regulation, epithelial barrier function and cell motility. Deregulations of these processes lead to tumor initiation, promotion, and advancement [28, 31]. We revealed that breast cancer females who carried AhR GG and PvuII TT genotypes had a better prognosis.

Conclusion

We conclude that the wild types of PvuII and AhR polymorphisms were related to better breast cancer prognosis and the mutant genotypes of PvuII and AhR polymorphisms were associated with poor breast cancer prognosis. Consequently, PvuII T/C and AhR G/A alleles may consider a prognostic factor in breast cancer progression.

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