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Association of *PIK3CA* and *HER2BB* genes' polymorphisms with breast cancer Iraqi patients

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ABSTRACT

Globally, breast cancer is the common malignancy affecting women and understanding its associated molecular events could help in disease prevention and management strategies. The present study was set to investigate an association between *PIK3CA* and *HER2BB* genes polymorphisms with breast cancer. For this purpose, 90 subjects were participated in this work, including 60 female patients diagnosed with breast cancer recruited from Oncology hospital, Baghdad - Iraq and 30 healthy women as a control group. Results: There was significant difference between genotype frequency of IIE391MET polymorphism for breast cancer and healthy controls. The heterozygote AG genotype IIE391MET (rs2230461) of *PIK3CA* seems to retain beneficial impact for the protection from breast cancer potential. While SNP homozygous TT and heterozygous CT genotypes (rs4855093 T >C SNP) *PIK3CA* seem to contribute to the predisposition of breast cancer development in the investigated set of patients. The SNP (rs1136201 A/G SNP) *HER2BB* genotyping results showed no association of the AG genotype with the risk of developing breast cancer. While homozygous AA genotype (rs1058808 C/A SNP) *HER2BB* seem to contribute to the susceptibility of breast cancer development in the investigated set of patients. In conclusion, we demonstrate that the *PIK3CA* and *HER2BB* SNPs have a correlation with the pathogenicity of breast cancer in Iraqi population.

Keywords*:* PIK3CA, HER2BB, Polymorphisms, Breast cancer

Introduction

Breast cancer is a life threatening malignancy that affects one in every eight women worldwide as stated by recent epidemiological statistics [1]. The high predicted global cancer statistics (12.5%) has put breast cancer as the second leading cause of cancer mortality in women with approximately 700,000 deaths in 2020 [2]. This emphasizes that much more efforts need to be allocated to understand the molecular events associated with breast cancer initiation and progression. The identified disease-associated biological changes could be assessed further for their potential to improve current patients' risk stratification, disease management and the development of novel therapeutics targets [3, 4].

The *PIK3CA* gene is situated on chromosome 3q26.3 and encompasses a region that includes 20 exons responsible for encoding the P110α protein. According to[5], a significant majority of *PIK3CA* mutations are situated in exons 9 and 20, which corresponds to the helical and kinase domains, respectively. *PIK3CA* is found to be the second most predominant gene mutations in breast cancer which the rate of its mutation is 16.4% to 45% [6]. Indeed, estrogen-receptor positive breast cancer cases showed to have significantly higher frequency of *PIK3CA* mutations reaches up to 40% [7].

The human epidermal growth factor 2 (*Her2*), alternatively referred to as *Her2-neu* or *ErbB2*, is a proto-oncogene that assumes to have a pivotal function in the signal transduction processes governing the normal growth and development of breast tissue. The *Her2* gene is responsible for the synthesis of the Her2 receptor protein. The aforementioned entity is a member of the human epidermal receptor (Her) family [8]. The amplification of the *Her2* oncogene in breast cancer has been found to contribute to genomic instability [9, 10]. According to Knight and his group 2015 [11], cellular proliferation and motility/migration are heightened as a result of genomic instability. These characteristics additionally contribute to the invasiveness of tumours and their ability to spread to other parts of the body, as documented by [12]. Furthermore, such genetic alterations showed to be associated with an increase in the formation of new blood vessels (angiogenesis) and a decrease in cell death [10].

The human genome contains a large number of genetic polymorphisms SNPs. SNPs are the most commonly studied molecular markers in genetic disease owing to their ubiquitous spread throughout the genome; in addition to their low cost when compared to other high throughput advanced techniques such as whole genome sequencing [13]. Studies showed that SNPs have impact in the development of different type’s cancers [14, 15]. Each SNP that has been known to date only have a small relative risk; but altogether, they can provide an accurate assessment of the risk of breast cancer in the general population [16]. It is reported that at least 94 common SNPs have associated with the risk of breast cancer [17]. SNPs can alter the encoded amino acids, be silent, or exist in noncoding sequences. They can cause disease by affecting promoter activity (gene expression), messenger RNA configuration (stability), and proteins. As a result, identifying and analyzing multiple gene variants may lead to a better understanding of their influence on gene function and an individual's health [18]. Activating oncogenic

mutations are often characterized by gain-of-function single-base alterations [19]. Locally in Iraq, breast cancer tops the list of women-effected malignancies and accounts for 22.2% of the diagnosed cases [20]. However, no previous study has investigated its association of *PIK3CA* and *HER2* SNPs with breast cancer.

Materials and Methods

Samples

A total of 90 peripheral blood samples were collected from 60 breast cancer patients and 30 from apparently healthy women, as a control group, during the period of April 2022 to Jun, 2023. The recruited breast cancer patients and their apparently healthy counterparts aged between 2070 years. Breast cancer patients had been diagnosed by specialist physicians at Oncology Teaching Hospital /Medical City/ Baghdad. Participants' demographic information and clinicopathological data were obtained from patients' hospital records and a questionnaire was prepared for the healthy controls. Informed consent was obtained from all participants enrolled in this study and the Biomedical Research Ethics Committee of Mustansiriyah University has approved this study (Reference No: BCSMU/0322/00028Z).

Molecular examination

Genomic DNA was extracted from 200 µl of each of the collected blood samples using a Rotor gene Q. Real-time PCR System (QIAGEN) was used to perform qPCR-HRM. While the sequence variations of *PIK3CA* and *HER2* genes were chosen to investigate their relationships with breast cancer patients. The SNPs detection (IIE391MET / rs2230461 A/G, M176I / rs4855093 C/T, Ile655Val / rs1136201 A/G, Pro1170Ala / rs1058808 C/A) were achieved by using HRM real-time PCR (Table 1).

Table 1: The primers sequence of *PIK3CA* and *HER2* genotypes

Primer	Sequence (5′→3′ direction)
*PIK*3CA (IIE391MET / rs2230461 A/G)	
Forward	AGGTGGAATGAATGGCTGAA
Reverse	GAGCAGCACGAGGAAGATCA
*PIK*3CA (M176I / rs4855093 C/T)	
Forward	GGTGAGTGAGTGGTGAGTGA
Reverse	TGCAGTGTTCATCAAGTCTACG
HER2 (Ile655Val / rs1136201 A/G)	
Forward	AATCCCTGACCCTGGCTTC
Reverse	CAAGACCACGACCAGCAG
*HER2 (*Pro1170Ala / rs1058808 C/A)	
Forward	CCTGCTGCCCGACCTG
Reverse	GACCCCATTCTTCCCTGG

For this assay, DNA samples both for breast cancer patients and healthy controls were genotyped for the above mentioned *PIK3CA* and *HER2* genes' four different SNPs. The q-PCR reaction mixture total volume was 20 µl composed 10µl of *2xTransStart*® Tip Green qPCR Super Mix, 1µl from each forward and reverse primer (10µM), 3µl of template DNA, and 5µl nuclease-free water. q-PCR amplification reaction was performed using a programmed thermocycler with one hold cycle of 94°C for 30 sec,

40 cycles of 94°C for 5 sec, 60°C for 15 sec, 72°C for 20 sec and (55-95) °C for 0.2 sec for 1 degree.

Statistical analysis

The differences in alleles /genotyping frequencies and significant between patients and controls were analyzed by the Fisher's exact test, P values less than 0.05, were considered significant. The strength of the association of disease with respect to a particular allele/genotypes expressed by odd ratio (OR), and Confidence Intervals (CI). It was calculated only for those alleles/genotypes which were increased or decreased in breast cancer patients as compared to the control group [].

Results and Discussion

PIK3CA rs2230461 A/G Polymorphisms

For this SNP analysis, the wild type genotype (AA) and allele (A) were designated as a reference. A total of 45% (27/60) of breast cancer cases were confirmed to be heterozygous (AG). While 33% (20/60) of breast cancer patients showed to have the homozygous (AA) and 22% (13/60) of them were found to be homozygous to the mutant genotype (GG). Frequencies only 27% (8/30) of the healthy controls group exhibited this genotype. These frequencies were differ when compared to those observed in the healthy controls (27 %(8/30), 53 %(16/30), and 20% (6/20), respectively. The odd ratio and % CI for the AG and GG genotypes were 2.7 (0.96 - 7.54), implying that this genotype (AG) could confer a risk for breast cancer development than the wild type AA (Table2).

Table 2: Genotype and allele frequencies analyzed by Hardy-Weinberg equilibrium of rs2230461 A/G *PIK3CA* gene polymorphism between breast cancer patients and healthy control groups

Groups		***PIK3CA* gene at position +175 (dbSNP-ID: rs2230461)**				
		Genotypes			**Alleles**	
		AA	**AG**	**GG**	***A***	***G***
Breast cancer patients (No. =60)	No.	(20)	(27)	(13)	(67)	(53)
	%	33 %	45 %	22 %	56 %	44 %
Healthy Controls (No. = 30)	No.	(16)	(8)	(6)	(40)	(20)
	%	53 %	27 %	20 %	67 %	33 %
OR		1.00	2.7	1.7	1.00	1.5
95% (C.I.)		(Reference)	(0.96 - 7.54)	(0.53-5.58)	(Reference)	(0.82-3.01)
P value		----	0.05*	0.3	----	0.1

***PIK*3CA rs4855093 C/T Polymorphisms**

For this genomic location, breast cancer patients showed a total frequency of 42% (25/60) for the heterozygous genotype(CT), 30% (18/60) were homozygous(CC) and 28% (17/60)of the patients were found to be homozygous mutant genotype (TT). The frequencies of these genotypes differ significantly from the control group; 16.6% (5/30), 66.6% (20/30), and 16.6% (5/30), respectively. The odd ratio and 95% CI for the CT and TT genotypes in rs4855093 polymorphism were 5.5 (1.75-17.58) and 3.6 (1.15-12.33), respectively, resulting in statistical significant differences (p= 0.003** and p=0.02*, respectively) between the compared groups (Table3). The OR and 95% CI for 59 T alleles were 2.9 (1.46 - 5.75); p = 0.002**, with significant difference from the control group, demonstrating that these genotypes could confer a higher risk of breast cancer development than the wild-type CC do.

Table 3: Genotype and allele frequencies assessed by Hardy-Weinberg Equilibrium of *PIK3CA* gene polymorphism rs4855093 between breast cancer patients and control group

Groups		***PIK3CA* gene at position +528 (dbSNP-ID: rs4855093)**				
		Genotypes			**Alleles**	
		CC	**CT**	**TT**	***C***	***T***
Breast cancer patients (No. =60)	No.	(18)	(25)	(17)	(61)	(59)
	%	30.0 %	42 %	28 %	51 %	49 %
Healthy controls (No. = 30)	No.	(20)	(5)	(5)	(45)	(15)
	%	66.6 %	16.6 %	16.6 %	75.0 %	25.0 %
OR		1.00	5.5	3.6	1.00	2.9
95% (C.I.)		(Reference)	(1.75-17.58)	(1.15-12.33)	(Reference)	(1.46-5.75)
P value		----	0.003**	0.02*	----	0.002**

rs1136201 A/G Polymorphisms *HER2*

The genotype and allele frequencies analysis for these genomic loci, rs1136201 A/G Polymorphisms *HER2*, of the breast cancer patients and healthy controls, are given in tables 4, AA and A were designated for both wild type genotype and allele (A) and used as a reference for this analysis. A total of 67% (40/60) of breast cancer patients were confirmed to be heterozygous (AG). 23% (14/60) with AA genotype and only 10% (6/60) of them were found to be homozygous for the mutant genotype (GG). The frequencies of these genotypes do not differ significantly from the control group ((63 %19/30,), (30 %9/30,), and (7 %2/30,), respectively). The odd ratio and % CI for the

AG and GG genotypes were 1.3 (0.49-3.67) and 1.9 (0.31-11.73), respectively. No statistically significant differences were found (p=0.5 and p=0.4) form this analysis suggesting that these observed genotypic variations do not have impact on the pathogenicity of breast cancer than the wild type AA in the studied set of patients. For G allele, the O.R and 95% CI was 1.2 (0.65-2.31); p=0.5 with no significant difference from the control (Table 4).

Table 4: Genotype and allele frequencies statistically analyzed by Hardy-Weinberg Equilibrium of *HER2* gene polymorphism rs1136201 A/G between breast cancer patients and control group

Groups		***HER2* gene at position + 655 (dbSNP-ID: rs1136201)**				
		Genotypes			**Alleles**	
		AA	**AG**	**GG**	***A***	***G***
Breast cancer patients (No. =60)	No.	(14)	(40)	(6)	(68)	(52)
	%	23 %	67 %	10 %	57 %	43 %
Healthy controls (No. = 30)	No.	(9)	(19)	(2)	(37)	(23)
	%	30 %	63.3 %	6.6 %	61.6 %	38.3 %
OR		1.00	1.3	1.9	1.00	1.2
95% (C.I.)		(Reference)	(0.49-3.67)	(0.31-11.73)	(Reference)	(0.65-2.31)
P value		----	0.5	0.4	----	0.5

rs1058808 C/A Polymorphisms *HER2*

In respect to the genotyping and allele frequency analyses for the rs1136201 in breast cancer patients compared to controls, 55% (33/60) of the breast cancer cases were confirmed to be heterozygous CA, 20% (12/60) of them were homozygous wild types (CC), and 25% (15/60) were found to be homozygous mutants (AA). This was not the case in the healthy controls where the frequencies of these genotypes differ significantly from those of the patients group ((60 % (18/30), (40 % 12/30), and (0 % 0/30,), respectively, Table 5). The odd ratio and 95% CI for the CA and AA genotypes in rs1136201 polymorphism were 1.8 (0.68-4.90) and 31 (1.66-57.68), respectively. These differences were statistically significant (p= 0.2 and p=0.02*, respectively, Table 5). The OR and 95% CI for 63 A alleles were 0.4 (0.24 - 0.91), with significant difference (p = 0.02), from the healthy controls, indicating that these genotypes associated with higher risk of breast cancer than the wild-type CC.

Table 5. Genotype and allele frequencies assessed by Hardy-Weinberg Equilibrium of *HER2* gene polymorphism rs1136201 between breast cancer patient and control group

Groups		***HER2* gene at position +528 (dbSNP-ID: rs1058808)**				
		Genotypes			**Alleles**	
		CC	**CA**	**AA**	***C***	***A***
Patients (No. =60)	No.	(12)	(33)	(15)	(57)	(63)
	%	20.0 %	55 %	25 %	47 %	53 %
Controls (No. = 30)	No.	(12)	(18)	(0)	(42)	(18)
	%	40 %	60 %	0 %	70 %	30 %
OR		1.00	1.8	31	1.00	0.4
95% (C.I.)		(Reference)	(0.68-4.90)	(1.66-57.68)	(Reference)	(0.24 - 0.91)
P value		----	0.2	0.02*	----	0.02*

DISCUSSION

In the current study, the *PIK3CA* I391M polymorphism was investigated in 60 women diagnosed with breast cancer patients and 30 age-matched healthy controls in order to verify the impact of *PIK3CA* variant on the risk of breast cancer development in Iraqi patients. Our results showed significant differences in the genotype frequencies of this genomic locus. AG genotype was significantly associated with the presence of breast cancer (P = 0.05, OR 2.7, 95% CI 0.96 -7.54). The rs2230461 is a missense polymorphism which causes the replacement of isoleucine to methionine at codon391. The rs2230461 and such these structural variants might be potential SNP markers for breast cancer pathogenicity.

In respect to the *PIK3CA* M176I polymorphism, our study results showed that the homozygous genotypes (CC and TT) and the heterozygous genotype (CT) exhibited discrepancy in the relationship and correlation between breast cancer patients and healthy controls. It is also showed that there is an association of the CT genotype (with a relatively greater degree of significantly and higher frequency percentage than the AG genotype) with the risk of developing breast cancer. Thus both genotypes can be investigated further for their utility as a genetic indicators associated with breast cancer pathogenicity. The results also showed that the homozygous genotype TT is significantly associated with the risk of developing the breast cancer.

Genetic imbalance in this locus (rs4855093) has been recently linked to breast cancer development [21]. Such genetic variation is believed to contribute to the widespread allelic expression imbalance between *PIK3CA* missense mutant and wild-type alleles in breast cancers, predominantly towards the preferential expression of the mutant

allele. Considering the very little known about this SNP and the lack of local studies that investigating its association with malignancies, the promising finding of our study could add a new insight to the potential involvement of rs4855093 in breast cancer pathogenicity.

Allelic expression imbalance of *PIK3CA* mutations is frequent in breast cancer and prognostically significant [21]. Mutations that are located far from the active site and which increase the enzymatic activity are associated with changes in the dynamic behavior of the protein in a large-scale. In other words, these mutations have allosteric impacts on PIK3CA protein [22]. A significant connection between the PIK3CA and age in women with breast cancer has been reported.

Regarding for I655V polymorphisms, the findings of the present study showed that the homozygous genotypes (AA and GG) and the heterozygous genotype (AG) have an analogic in the relationship and correlation between breast cancer patients and healthy controls. It is also showed that there is no association of the AG genotype with the risk of developing breast cancer in the studied subjects. The results also showed that the homozygous genotype GG is less associated with the risk of developing the disease.

The results of our study agreed with that of Han and his group, who showed that SNP not differed significantly between breast cancer patients and healthy control woman[23]. A weak association between I655Val (G) allele and increased breast cancer risk in Caucasian populations, as well as between the Val/Val (G/G) genotype and elevated breast cancer risk in Austrian women was assumed [23].

Furthermore, Pro1170Ala polymorphisms demonstrated that the homozygous genotypes (CC and AA) and the heterozygous genotype (CA) showed a discrepancy in the relationship and correlation between breast cancer patients and healthy controls. It is also showed that there is no association of the CA genotype with the risk of developing breast cancer. The results also showed that the homozygous genotype AA is significantly associated with the risk of developing the breast cancer.

ERBB2 rs1058808 loci were associated with the risk of epithelial ovarian cancer (EOC) [24]. Under the codominant model, rs1058808 polymorphisms associated with HER2 protein expression in breast cancer (p=0.007; p=0.008, respectively). For SNP rs1058808, patients with genotypes CG and GG were more likely to have high HER2 protein expression than patients with genotype CC (p=0.007) [25]. *HER2* rs1058808 may be utilized as genetic screening markers for predicting colorectal cancer, and gastric cancer [26]. *HER2* rs1058808 also recently linked to the susceptibility of metastatic breast cancer [27].

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