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Genetic variant PIK3CA rs7640662 (C/G) single nucleotide polymorphism Gene of Breast Cancer Patients in Iraqi

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Abstract:

Background: Phosphatidylinositol-3-kinase (PI3K) is a group of enzymes involved in cellular growth, proliferation, differentiation, cell motility,intracellular trafficking, and survival that play very important roles in developing breast cancer *PIK3CA* is a gene that encodes α catalytic subunit of this enzyme.

A common polymorphism of *PIK3CA*, rs7640662 (C/G), was analyzed, and its association to breast cancer cases was determined Tetra-primer amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR) method was performed to genotype rs7640662.

Participants in the study comprised 50 girls with sporadic breast cancer, 30 girls with a first-degree cousin who had the disease, and 30 girls in health.

In conclusion, the results of this investigation demonstrated that of the aforementioned founder mutations had been identified in the agencies analyzed. According to our research, breast cancer patients in Iraq have more frequent mutations in PIK3CA.

Keywords: PIK3CA , breast cancer , PCR(T-ARMS-PCR)

INTRODUCTION

Phosphatidylinositol-3-kinase (PI3K) is a family of enzymes that has been shown to involve in many aspects of cell growth and survival in many cases of breast cancer [1]. The pathway is crucial for tumor survival when tumors are under diminished amount of nutrient and pressure of oxygen [2]. Hereditary factors account for roughly 5–10% of all incidences of breast cancer with an early onset. It has been found that PIK3CA gene was mutated in breast cancer [3]. Most of these mutations have been localized to hotspots in exons 9 and 20 of the PIK- 3CA gene, and their nature seems to be oncogenic [4] PIK3CA mutations and PTEN loss (a tumor suppressor that normally inactivate PI3K) coexist in breast cancers. In the breast, the observed frequency of tumors with coexisting PTEN loss and PIK3CA mutations is 8.7% [5]. The rs7640662 (C/G) is an intron variant of the PIK3CA gene located on chromosome 3, location 3: 179184213.

METHOD AND MATERIALS

The Margan Hospital for Research in Applied and Experimental Medicine at the National University of Science once hosted the authors' study. The PIK3CA gene mutational screening in cases of breast cancer, both hereditary and sporadic, was the main focus of this investigation. In order to search for the : PIK3CA gene, all exonintron boundaries have been sequenced. In the past, the PIK3CA exon-intron boundaries were amplified by polymerase chain reaction (PCR) using oligonucleotide primers made from the gene's intronic regions.

Sampling and document gathering

In this experiment, 60 samples were protected, of which 30 were classified as routine samples and 30 as samples from patients who had breast cancer complaints (control group). The patients ranged in age from 26 to 70. Three to five milliliters of blood were drawn into vacutainer tubes with EDTA acting as the anticoagulant in order to extract the DNA and carry out the subsequent PCR analysis. After being transferred, the blood samples were stored at -20 °C in the Laboratory of Genetic Engineering Department of the Biotechnology Research Center until needed.

DNA extraction

DNA genes were previously extracted using blooding and EDTA tubes from all things using a Mini Kit (FAVORGENE). The amount of pure (ng/ml) DNA that is eliminated has been calculated at 260 nm and 280 nm using a NanoDrop of spectrophotometer (OPTIZENn POP – Korea).

Genotyping

White blood cells (WBCs) were utilized to extract genomic DNA using the (Favrogene) DNA extraction kit for both diabetes and management organizations. selected rs7640662

Forward	inner	ATTGGTGGAGTCCATTTACACCTTCACC	125
Reverse	inner	TGGTGATTCTGCCAATACTTATAGGCTTAC	237
Forward	outer	ATTATTGGCTAGTGCCTATTTTCACAGCA	304
Reverse	outer	GGCTGTTGCAAGGACAATATTTTCAAA	

Polymerase chain reaction (PCR) was performed with the following cycle conditions: initial denaturation at 95 °C for 5 min, 33 cycles of denaturation (95 °C for 30 s), annealing (58 °C for 30 s), extension (72 °C for 40 s), and a final extension step (72 °C for 10 min). PCR products were analyzed by gel electrophoresis in 3% agarose and visualized by ethidium bromide staining. The product sizes for detection of the rs7640662 polymorphism were 125 bp for the C allele, 237 bp for the G allele, and 304 bp for the non-allele specific primers (control band). The multiplex tetra-primer amplification refractory mutation system polymerase chain reaction (ARMS-PCR) method [6] was used to analyze the *PIK3CA* gene SNP. In this method, four primers (two primers as outer primers and two primers as inner primers) were used to amplify the rs7640662 SNP with 125 bp and 237 bp amplicons *(Table I)*.

Results AND DISCUSSION

Recent analysis results reveal that the DNA has (50-200)ng and righteousness (1.7-2.2), as shown in parent (1) Numerous studies have been made regarding its genotyping, and the PIK3CA gene used to be associated with risky development in higher examiners. It was once suggested that existing tests examine genotyping PIK3CA similarly to contaminated DNA because of these findings.

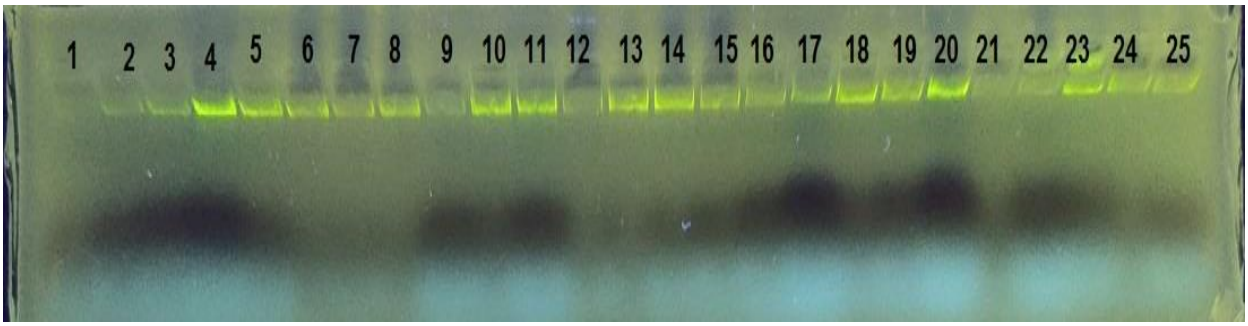


Figure .2 Electrophoresis example of gnomic DNA in study gatherings, path 1-17 DNA from patients path 18-25 DNA from control.

PIK3CA gene Polymorphism

Table (1) shows the distribution of PIK3CA gene in control and patients Breast cancer. CG 46%, CC 34% and GG 20% in controls and 22%, CG 42% and CC 36% in cases. An increased frequency of homozygotic mutant genotypes (CG) were found in patients compared to controls. There was a statistically significant difference in the distribution of allele frequencies in cases and controls (W v/s M: $\chi 2$ P<0.0001, OR 0.5149, 95% CI 0.5149 (0.2958 to 0.8961) .

Table (1): Genotype and allele distribution PIK3CA gene polymorphism in patient and control, shown the Odd Ratio value.

Genotypes	Patients (N=30)		Control (N=30)	OR(95%CI)	P-value
PIK3CA	C/C [a], n(%)	7(23%)	6 (20%)		
	C/G, n(%)	18(60%)	11 (36%)	0.6900 (0.3332 to 1.4288)	0.3
	G /G, n(%)	5 (1%)	13 (43%)	49.4500 (6.2321 to 392.3697)	0.002*

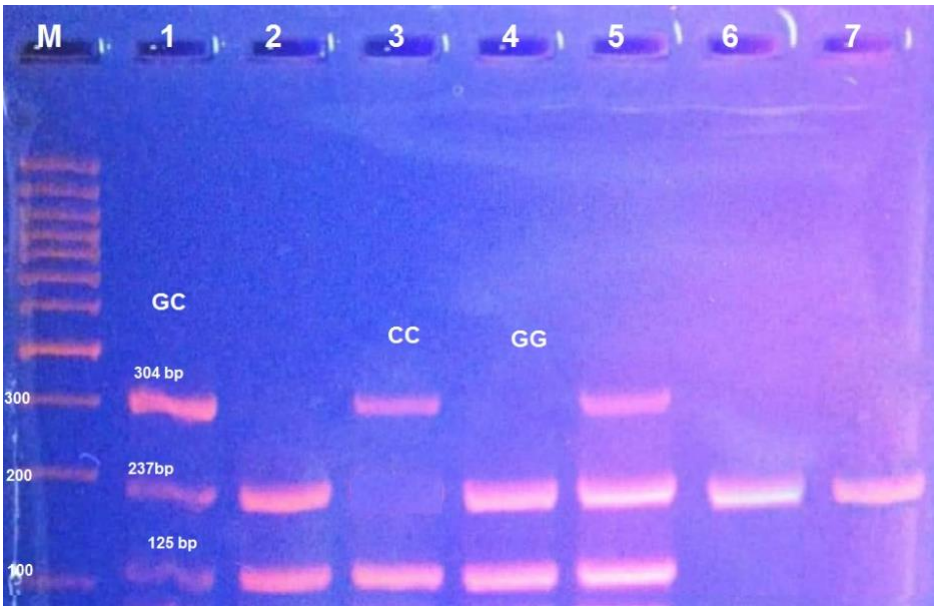
Allele Frequency					
	C, **n (%)**	53 (0.53)	38 (0.38)	1.5006 (0.8398 to 2.6813	0.17
	G, **n (%)**	46 (0.46)	61 (0.61)		

GG-homozygous wild , GC-heterozygous, CC-homozygous mutant

P<0.005: OR =(95%Cl): [a] Reference

rs7640662 was not associated with the risk of breast cancer in this Persian population; however, it was observed that heterozygote (GC) is the most common genotypes in both case and control samples.

Breast cancer has numerous numbers of molecular markers that can be targeted by specific drugs [7] Alteration in genotype may affect the response of patient to chemotherapy and may show a wide range of resistance [8]. Genetic variations in the PI3K–PTEN–AKT–mTOR pathway affect clinical outcomes in patients treated with certain types of drugs [9]. The frequency, breast cancer subtype specificity, and signaling effects of *PIK3CA*, *AKT*, and *PTEN* mutations in human breast tumors and breast cancer cell lines have been studied [10]. It has been shown that *PIK3CA* pathway aberrations are common in breast cancer [11]



Figure(1):lines(1,5) have GC genotype, and (b) GG (2,4,6,7) and CC (3) genotypes are shown by lines 1 and 2, respectively . On a 2% agarose gel, electrophoresis was carried out using 5-8 V/cm for 1 hours. There was a DNA molecular marker in Lane M. (100 bp).

CONCLUSION

Breast cancer has numerous numbers of molecular markers that can be targeted by specific drugs [12]. Alteration in genotype may affect the response of patient to chemotherapy and may show a wide range of resistance [13]. Genetic variations in the PI3K–PTEN– AKT–mTOR pathway affect clinical outcomes in patients treated with certain types of drugs [14]. PIK3CA mutations primarily occurred at hotspots in exons 9 and 20 that encode portions of the helical and kinase domains of PI3K have been reported to be associated with approximately one third of breast cancers [15]. These mutations have been reported to activate AKT and downstream signaling in model systems [16].

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