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Association Between the PIK3CA Ile391Met Polymorphism and the Risk of Breast Cancer in an Iranian Population

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Abstract

Introduction: Breast cancer, as a multifactorial disease is the most frequent cancer among women and second most commonly diagnosed cancer in worldwide. Breast cancer is associated with mutations in several genes such as *PIK3CA*. Phosphoinositide 3 kinase (PI3K) is an important group of lipid kinases that regulate the vital cellular functions such as survival, proliferation, cell growth, motility, differentiation, and intracellular trafficking**.** The aim of this study is to evaluate the association of rs2230461 of *PIK3CA* gene with the incidence of breast cancer.

Materials and Methods: A total of 198 healthy donors and 205 breast cancer patients were recruited. Genomic DNA was extracted from peripheral blood leukocytes by Triton X100 technique. Genotyping was performed using RFLP-PCR protocol. Chi-square test, odds ratios (ORs) and 95% CIs were used to determine associations.

Results: There were no significant differences observed regarding the *PIK3CA* genotype frequencies at codon 391 between patient and control groups ($P=0.17$). However, by comparing stage III breast cancer patients and control groups, there was a significantly higher frequency of the GG genotype among stage III cases compared to control ($P=0.01$). Although the PIK3CA I391M polymorphism has been located in the C2 domain and doesn't involve in the binding site, it can affect the protein function.

Conclusions: Since even those mutations that are far from the binding site can affect the protein function and change its dynamic behavior through allosteric impacts and lead to tumorigenesis at last. Since *PIK3CA* mutations mainly appear late in tumorigenesis, exactly before or coincident with invasion, and may be involved in tumor formation, it is suggested that this polymorphism may be involved in breast cancer invasion.

Keywords: *PIK3CA*, Polymorphism, Breast Cancer

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Introduction

Breast cancer, as a multifactorial disease is the most frequent cancer among women and second most commonly diagnosed cancer in worldwide. According to reports from the International Agency for Research on Cancer (IARC), there are 1.67 million breast cancer diagnosed in 2012 (25% of all cancers) with about 522 000 death reports.[1,2] The incidence of breast cancer in the last 30 years has been up trended in many countries. Epidemiologic studies suggest the important role of environmental and genetic factors in the development of breast cancer.[3,4] The incidence rates of breast cancer in all over the world reported as follows: highest in North America (age-standardized rate [ASR]: 123.6 per 100 000)[5] and Western Europe (ASR: 84.6 per 100 000),[3] intermediate in the Mediterranean and South American countries (ASR: 46 per 100 000)[4] and lowest in Southeast (ASR: 25.5 per 100 000)[4,6] and South Central Asia (ASR: 21.8 per 100 000).[6] Breast cancer is ranked first among malignancies in Iranian women,[7,8] comprising 24.4% of all cancers[9] with a crude incidence rate of 17.81 and an ASR of 23.65 in the year of 2006.[8] According to the new statistic, 6160 breast cancers are diagnosed in Iran each year, which 1063 cases of them lead to death.[10,11] According to the report of World Health Organization (WHO), the incidence of breast cancer has annually increased by 2%.[12] A portion of this increase is related to changes in reproductive patterns, such as delayed childbearing and having fewer children.[13] The crude rate and ASR of breast cancer incidence in the Iranian women were increased respectively from 12.19 and 15.96 in 2003 to 22.09 and 28.25 in 2009 per 100 000.[14] Although the incidence of breast cancer in Asian women is lower than women in Western countries, its incidence trend is higher than Western countries. Breast cancer is happening

in the Iranian women at least a decade earlier than women in developed countries and most of the patients are in the age of 40–49 years old in Iran.[15] Breast cancer has four stages. The stage 0 are non-invasive cancers that remain in initial location and stage IV are invasive cancers that have spread to other parts of the body.[16] With the completion of the Human Genome Project (HGP), single nucleotide polymorphisms (SNPs) regarded as an essential factor in the development of different cancers.[17,18] Each SNP that has been known to date only have a small relative risk on their own, but altogether, they can provide an accurate assessment of the risk of breast cancer in the general population.[19] It is reported that at least 94 common breast risk SNPs have associated with breast cancer.[20] Breast cancer is associated with mutations in several genes such as *BRCA1, BRCA2, CDH1, PIK3CA* and *TP53*.[21] Phosphoinositide 3 kinase (PI3K) is an important group of lipid kinases that regulate the vital cellular functions such as survival, proliferation, cell growth, motility, differentiation, and intracellular trafficking. PI3K is divided into 3 classes (I–III) based on their primary structure and lipid substrate specificity. Class I PI3K are heterodimers that further classified into class IA and IB. Among all *PI3K* classes, class IA has the most closely implicated in cancer and comprise a p110 catalytic subunit and a p85 regulatory subunit. P110α (encoded by *PIK3CA*), is one of the 3 isoforms of p110 subunit.[22] After the *TP53*, *PIK3CA* is found to be the second most predominant gene with mutations in breast cancer which the rate of its mutation is 16.4% to 45%.[21,23] PI3K/Akt/mammalian target of rapamycin (mTOR) is a main intracellular signaling pathway, which is frequently activated in breast cancer. PI3K/Akt/mTOR responds to the accessibility of hormones, nutrients, and growth factor stimulation. The central role of this pathway in tumor cell growth and proliferation is played by Class IA *PI3K*.[24] Mutations in the *PIK3CA* affects downstream pathways. So that they are often causing the dysregulate of PI3K/AKT/mTOR signaling pathway and have been reported in various human cancers such as breast cancer.[25]

The aim of this study is to evaluate the association of rs2230461 of *PIK3CA* gene with the incidence of breast cancer. The rs2230461 is a missense polymorphism which causes the replacement of isoleucine to methionine at codon 391. To the best of our knowledge, this is the first time that the association of rs2230461 with breast cancer incidence has been studied.

Materials and Methods

Sampling

Peripheral blood samples were collected from 198 healthy donors, aged between 35 and 55 years old (control cases) and 205 breast cancer patients. All volunteers, who met the inclusion criteria for participating in this study, were Iranian who resided in Guilan province. The ethics approval was performed based on the Iranian ministry of health and medical education criteria. To prevent from any biased sampling, all samples were collected randomly, without any discriminatory information about her families, social and histopathological background of the subject. Normal blood samples were collected from women examined at Razi hospital, Rasht, Iran.

Genomic DNA Isolation

DNA was extracted from peripheral blood leukocytes by Triton X100 technique. The extracted DNA was diluted in 100 µL deionized water and it was kept at -20˚C until use.

Genotyping

Initially, the upstream and downstream flanking regions of rs2230461 were taken from National Center for Biotechnology Information (NCBI). RFLP PCR primers were designed by using the Oligo7 software. To avoid the formation of stable primer-dimers, the primers were accurately assessed by NCBI/Primer-BLAST online software. The forward and reverse primer sequences are 5′ ATAACCTTACCACCCCTT 3′ and 5′ AGCGGTATAATCAGGAGT 3′ respectively. RFLP PCR was performed using about 40 ng genomic DNA, 2 pmol of each primer and 7.5 µL of Ampliqon Taq DNA Polymerase Master Mix in a total volume of 15 µL. After performing the gradient temperature, the best-optimized condition was as follow: the initial denaturation step at 95°C for 5 minutes, 30 cycles constituting the denaturation in 95°C for 30 seconds, annealing in 56°C for 30 seconds, the elongation in 72°C for 30 seconds, followed by a final elongation step at 72°C for 5 minutes. The length of PCR product was 451 bp which separated using 2% agarose gel electrophoresis and safe stain staining.

After ensuring sample amplifications on the agarose gel, the 451 bp PCR products were digested using 1 unit of BsrG1 restriction enzyme (Thermo Scientific, American) at 37°C for 2 hours. To determine the genotypes, digested PCR products were separated using 2% agarose gel electrophoresis and safe stain staining once again. The wild-type allele (Ile), which has no BsrG1 restriction enzyme site, revealed as a single fragment of 451 bp and is indicative of the homozygous wild-type (AA) genotype, while the homozygous mutant genotype (GG) generates two fragments of 313 and 138 bp, and lastly the heterozygous genotype (AG) contains all 3 fragments of 451, 313 and 138 bp. To confirm the accuracy of genotyping results, twenty samples including 15 homozygotes and 5 heterozygotes, were randomly selected and re-genotyped using the same method.

Statistical Analysis

The allele frequency within each group was determined as the number of occurrences of an individual allele divided by the total number of alleles. To determine whether any significant differences in polymorphism frequencies occurred between the case and control population, allele and genotype frequencies were compared using the chi-square method and the MedCalc version 9.6.4.0. Odds ratio (OR) and 95% CIs were calculated to determine the risk of breast cancer associated with a given *PIK3CA* genotype. *P* values of less than or equal to 0.05 were considered to represent statistical significance.

Ethical Considerations

All individuals participated in the current study were consented in a process approved by the Ethics Committee for Human Genome/Gene Research at the University of Guilan

(NO. IR.BMSU.REC.1396.443).

Results

Participants Information

This case-control study included 198 breast cancer patients and 205 healthy individuals. The breast cancer patients' age ranged between 35 and 55 years.

Genotyping

A partial fragment of the *PIK3CA* gene that contained SNP rs2230461was amplified using by PCR. A PCR product of 451 bp was detected by running aliquots of the PCR product on 2% agarose gel electrophoresis. No enzymatic restriction occurred in AA genotype and a 451bp fragment was obtained. The 313 and 138 base pair bands were seen in GG genotype and 313, 138 and 451 base pair bands were obtained in AG genotype (Figure 1). Also, the frequency of the rs2230461 polymorphism of *PIK3CA* gene was analyzed.

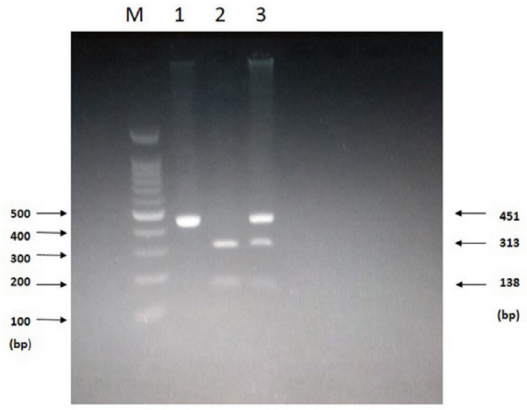

Figure 1. RFLP Analysis of the Polymorphism. Lane 1: undigested PCR product of 451 Ile/Ile genotype, lane 2: two fragments of 313 and 138 bp for Met/Met genotype, lanes 3: three fragments of 451,313 and 138 bp for Ile/Met genotype. M: molecular size marker.

Data Analysis

All information about allele and genotype frequencies and related ORs (95% CI) for patients and controls have been shown in Table 1. There were no significant differences observed regarding the *PIK3CA* genotype frequencies at codon 391 between patient and control groups ($P=0.17$). However, a genotype-phenotype subanalysis of cancer stages was done on 52 out of 195 breast cancer patients who were at stage III. by comparing stage III breast cancer patients and control groups, there was a significantly higher frequency of the GG genotype among stage III cases compared to control ($P=0.01$). (table is not shown). Moreover, AG genotype seems to be the risk factor in a co-dominant model of hereditary between case and control groups ($P=0.006$, OR 4.3, 95% CI 1.5-12.24).

Discussion

PI3K is an important group of lipid kinases that regulate the vital cellular functions such as proliferation, cell growth, motility, differentiation, and intracellular trafficking. Also, previous studies have shown that *PIK3CA* is a key factor in the survival of tumor cells.[22] PI3K/Akt/ mammalian target of rapamycin (mTOR) is a main intracellular signaling pathway, which is frequently activated in breast cancer. PI3K/Akt/ mTOR responds to the accessibility of hormones, nutrients, and growth factor stimulation.[24] To the best of our knowledge, this is the first study on the PIK3CA I391M polymorphism and the risk of breast cancer incidence. The PI3K/AKT signaling pathway was frequently activated by gene mutations.[26] Moreover, genetic polymorphisms of *PIK3CA* were also reported to be related with breast cancer, head-neck squamous cell carcinoma, esophageal squamous cell carcinoma, non-small cell lung cancer, endometrial cancer, gastric cancer and rectal cancer.[27-33] PI3K comprise two major subunits, a catalytic subunit p110 and a regulatory subunit p85.[34] The regulatory subunit has two major roles: they stabilize the catalytic subunit against thermal denaturation, and they maintain the catalytic subunit in an inhibited, low activity state.[35,36] p85 and p110 are both multidomain proteins that bind to each other and to upstream activators.[37] C2-iSH2 is a communication interface that is required for inhibition of p110α by p85. Mutations that particularly disrupt the C2-iSH2 contact, led to loss of p110α inhibition. Even those PI3Kα 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 the protein.[38-41] A significant connection between the *PIK3CA* and association of age in women with breast cancer has been reported.[34] Specifically, mutations in the catalytic subunit, upregulate PI3K/AKT/mTOR pathway and promote carcinogenesis providing a way for the development

Table 1. Genotype Frequencies of PIK3CA Ile391Met Polymorphism Among Breast Cancer Cases and Controls

Genetic Models	Genotype	Patients No. (%)	Controls No. (%)	OR (95%CI)	*P* Value
PIK3CA	Ile/Ile	36	117	1.00	
Ile391Met	Ile/Met	8	9	4.3(1.5-12.24)	0.006
Codominant	Met/Met	8	22	0.98(0.42-2.30	0.97
Dominant	Ile/Ile	36	117	1.00	
	Ile/Met+Met/Met	16	31	1.60(0.81-3.15)	0.17
Recessive	Ile/Met+Ile/Ile	44	126	1.00	
	Met/Met	8	22	0.55(0.24-1.26)	0.16
Overdominant	Ile/Ile+Met/Met	44	139	1.00	
	Ile/Met	8	9	4.3(1.53-12.13)	0.005

of PI3K inhibitors toward cancer therapy. Even on the basis of mutations severity, several drugs were designed to inhibit PI3K–AKT–mTOR pathway for cancer therapy.[42]

In a previous study, Karakas et al studied the prevalence of *PIK3CA* mutations and the SNP rs17849073 in Arab breast cancer patients among 81 breast cancer tissues and 189 blood sample of the healthy donors. They found a total of 21 *PIK3CA* missense mutations with 25.9% frequency. *PIK3CA* mutations were significantly associated with lower grade and hormone receptor positivity. Also, they identified a high prevalence of the SNP rs7849073 in the Arab breast cancer population compared to the healthy group. Karakas et al showed the importance of *PIK3CA* mutation and polymorphism in susceptibility to breast cancer incidence.[43] In another study, Mir et al investigated the *PIK3CA* rs7640662 polymorphism association with breast cancer in a Persian population with 278 breast cancer patients and 128 healthy women. Mir et al did not observe any significant association between the rs7640662 and breast cancer incidence ($P>0.05$). However, their results showed no association between the rs7640662 and breast cancer incidence further research is needed based on larger sample size and in different ethnic population to realize the impact of *PIK3CA* rs7640662 on breast cancer incidence.[44]

In the current study, the PIK3CA I391M polymorphism was investigated in a series of 205 breast cancer patients and 198 population matched controls in order to verify the impact of *PIK3CA* variant on the risk of tumor development in Iranian breast cancer patients. Our results showed significant differences in the genotype distribution of the PIK3CA I391M polymorphism between stage III breast cancer patients and controls ($P=0.01$). AG genotype was significantly associated with the presence of breast cancer ($P=0.006$, OR 4.3, 95% CI 1.5-12.24). Although the PIK3CA I391M polymorphism has been located in the C2 domain and doesn't involve in the binding site, it can affect the protein function and finally lead to cancer. Since even those mutations that are far from the binding site can affect the protein function and change its dynamic behavior. On the other hand, a study has revealed that *PIK3CA* mutations mainly appear late in tumorigenesis, exactly before or coincident with invasion and demonstrating that *PIK3CA* may be closely related to the invasiveness of cancer cells. Furthermore, *PIK3CA* polymorphisms caused improvement of the PI3K signaling pathway in tumor tissues, that likewise suggested PIK3CA may be involved in tumor formation.[27,30] Since in our study, the PIK3CA I391M polymorphism, had only a significant relation with stage III breast cancer, it is suggested that this polymorphism may be involved in breast cancer invasion.

Conclusions

To the best of our knowledge, this case-control study for the first time studied the association of PIK3CA I391M polymorphism and susceptibility to breast cancer. In this population based on the breast cancer in Guilan, Iran, it has been demonstrated that the genetic polymorphism in *PIK3CA* gene (rs2230461 A>G) is not associated with the risk of breast cancer incidence. However, significant differences observed in stage III patients can be a molecular sign that reveals the *PIK3CA* rs2230461 may be closely related to the beginning of invasion in breast cancer cells. To make ensure, it is suggested that these structural variants in other populations with larger sample size to be investigated too. In addition, the rs2230461 and such these structural variants might be potential SNP markers for tumorigenesis of breast cancer which suggest genotyping of structural variants in drug designing and prescription of related drugs according to personal genotypes.

Authors' Contributions

All Authors contributed equally to this research.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

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