PIK3CA rs7640662 (C/G) single nucleotide polymorphism lacks association with breast cancer cases in Persians

ATEFEH MIR¹, MAHDIYEH HARATI SADEGH¹, ZAHRA AHMADINIA², PARHAM JABBARZADEH KABOLI³,*

¹Department of Biology, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan, Iran
²Department of Biology, Qom Branch, Islamic Azad University, Qom, Iran
³Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, UPM, Serdang, Selangor 43400, Malaysia

*Corresponding author: Parham Jabbarzadeh Kaboli; Phone: +60 1123749520; E-mail: researchgroups@drugdiscovery.ir

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Abstract: 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. In this study, DNA was extracted from peripheral blood samples of 278 women suffering from breast cancer and 128 healthy women. Tetra-primer amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR) method was performed to genotype rs7640662. P values and ODD ratios were measured using SPSS. P value less than 0.05 and ODD ratios more than 1 were considered as significant. All ODD ratios were less than 1, and P values were more than 0.05 showing that rs7640662 (C/G) and breast cancer are not significantly associated. However, the genotypes observed in the Persian population, as an ancient population living in the Middle East, was significantly different from the genotypes reported by HapMap for Asian populations. As a conclusion, rs7640662 was not associated with the risk of breast cancer in a Persian population; however, it was observed that heterozygote (GC) is the most common genotypes in both case and control samples.

Keywords: breast cancer, rs7640662, PIK3CA, single nucleotide polymorphism, ARMS-PCR

Introduction

Breast cancer is the most frequently diagnosed cancer in women and is the second most common cancer worldwide. Based on the GLOBOCAN database (2012), 25% (1.67 million) of all new cancer cases and 15% (522,000) of all cancer deaths in women were due to breast cancer [1]. 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 [2, 8]. The pathway is crucial for tumor survival when tumors are under diminished amount of nutrient and pressure of oxygen [4].

PI3Ks are heterodimeric molecules composed of a regulatory subunit and a catalytic subunit, which is divided into two different classes: IA and IB. Class IA PI3K catalytic subunit consists of a p110 catalytic subunit and p85 regulatory subunit [5]. There are three variants of the p110 catalytic subunit, one of which is p110α. PIK3CA encodes p110α of PI3K and is expressed in all cells [6–8]. The frequently distributed PIK3CA gene mutations in the common human cancers show that alterations of lipid kinase pathway by PIK3CA mutations contribute to the development of human cancers [9]. It has been found that PIK3CA gene was mutated in breast cancer [10–13]. Most of these mutations have been localized to hotspots in exons 9 and 20 of the PIK3CA gene, and their nature seems to be oncogenic [14] due to promotion of cell growth and invasion [12]. 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% [15]. The rs7640662 (C/G) is an intron variant of the PIK3CA gene located on chromosome 3, location 3: 179184213. According to the Reference SNP (refSNP)
Cluster Report of rs7640662, all genotypes reported for Asian rs7640662 (C/G)s are from Chinese [16].

Heterogeneity in response to chemoradiotherapy may be due to several factors, including age, sex, ethnicity, and drug–drug interactions. In addition, genetic variations in pharmacokinetic, pharmacodynamics, and drug action pathways have been shown to be important in determining sensitivity or resistance to treatment. Thus, one strategy to increase the effectiveness of chemoradiotherapy is to gain a better understanding of the influence a patient’s genetic background has on response to treatment [17]. It has been previously reported that genetic variations in several drug action pathways were associated with variation in clinical outcome of some cancer cases [18].

The variations of rs7640662 (C/G) has been studied as an important single nucleotide polymorphism (SNP) located in an important signaling enzyme gene (PIK3CA) related to some kind of cancer (e.g., esophageal cancer) [17, 18]; however, the association of this key SNP with breast cancer has not been studied to date. This study aimed to screen for rs7640662 (C/G) in breast cancer case and control samples of Middle Eastern populations (Persians and Arabs). Comparison of rs7640662 (C/G) genotypes between the studied subjects and the genotypes for populations of different origins was reported. The results of this research are important to show why some people of this subcontinent area do not respond to chemotherapy and their drugs may be regionally personalized, and it can also show whether Persians are most similar to eastern Asians or Europeans.

Materials and Methods

Sampling

In this study, the samples were collected from whole blood of healthy female subjects and females suffering from breast cancer from educational hospital, Zahedan University of medical sciences, Iran. A total of 128 control samples were selected from different ages (18–61), and 278 breast cancer cases were selected from females older than the age of 48 (48–64). According to Iranian medical ethics, all samples were prepared from the hospital.

DNA isolation and amplification

DNA was extracted from whole blood cells by phenol-chloroform protocol [19]. The multiplex tetra-primer amplification refractory mutation system polymerase chain reaction (ARMS-PCR) method [20] 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). PCR reactions were prepared using Taq DNA Polymerase 2× Master Mix Red (Ampliqon, Denmark) according to manufacturer's manual. 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).

Statistical analysis

χ² test was used to examine the differences in allele frequencies and distribution of genotypes between case and control samples using Statistical Package for the Social Sciences (SPSS) version 21. The association between genotype and risk of breast cancer was estimated by calculating the odds ratio (OR) and their 95% confidence interval (95% CI) with logistic regression models. All statistical tests were two-sided, and the P value < 0.05 and OR > 1 were considered significant, showing the effectiveness of allele distribution on the risk of breast cancer.

Results

In this study, we recruited 278 breast cancer cases and 128 healthy individuals. As it can be clearly seen, the number of control samples is approximately half of the number of cases because sample sizes were limited by the number of well-characterized clinical samples and
the number of healthy people who agreed to give their blood samples was limited in the area of interest; however, minor allele frequencies (MAFs) higher than 5% were targeted by HapMap projects. Due to the small number of control samples, first, it was needed to qualify the sample sizes whether sample sizes for both case and control samples are enough to continue the study or not. Therefore, the power of the estimation of this study was calculated and then the experiment was started. The statistical power was calculated based on the sample sizes available for case and control samples (278 and 128, respectively), desired significant level (5%), and minor allele frequencies for case and control samples (15% and 5%, respectively) [21–24]. SPSS version 21 was used, and calculated power was 88% which showed that the type II error is as low as 12%. Then, the research was continued. While type I error or $\alpha$ value decreases, type II error or $\beta$ value increases. According to Daniel and Cross (2013) [25], $\beta$ values less than 20% and $\alpha$ values less than 5% were considered significant. The statistical power of an experiment is $1 - \beta$ which was equal to 88%. This showed that the $\beta$ value is as less as 12% (<20%) for the sample sizes 278 and 128 for case and control samples, respectively. In addition, this calculation verified that the number of control sample is good enough to continue the SNP analysis in this research.

After running the T-ARMS-PCR, the results showed that 272 patients of 278 total cases had CG genotype (97.85%) and only 6 of them were CC genotype (2.15%), and also, GG genotype was not observed in this study. In the control group, all of the samples were CG genotype, and CC and GG genotypes were not observed (Fig. 1). Table II shows the genotype frequencies of rs7640662 SNP in both case and control samples. The nonconditional logistic regression analysis found no statistically significant differences between case and control samples in terms of distribution frequencies of SNP genotypes. Moreover, the SNP genotypes of patients and control samples were not significantly associated with breast cancer risk ($P$ value < 0.05).

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**Fig. 1.** PCR products. (a) Seven lines have GC genotype, and (b) GC (observed in both case and control samples) and CC (observed only in cases) genotypes are shown by lines 1 and 2, respectively.
than 0.05 and ODD ratios more than 1 were consid-
ered as significant. All ODD ratios were less than 1, and P
values were more than 0.05 showing that rs7640662
(C/G) and breast cancer are not significantly associated.
However, the genotypes observed in the Persian popula-
tion, as an ancient population living in the Middle East,
were significantly different from the genotypes reported
by HapMap project for Asian populations. rs7640662
was not associated with the risk of breast cancer in this
Persian population; however, it was observed that het-

Table II | Genotypic and allelic frequencies of rs7640662 (C/G) polymorphism in comparison between patient cases
and healthy subjects, and further information about blood donors

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Case N = 278</th>
<th>Control N = 128</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC (%)</td>
<td>6 (2.15)</td>
<td>0 (0.0)</td>
<td>1 (Ref)</td>
<td>0.094</td>
</tr>
<tr>
<td>CG (%)</td>
<td>272 (97.85)</td>
<td>128 (100)</td>
<td>0.163 (0.0091 to 2.9180)</td>
<td>0.217</td>
</tr>
<tr>
<td>GG (%)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0.461 (0.0091 to 23.3846)</td>
<td>0.699</td>
</tr>
<tr>
<td>CG + GG (%)</td>
<td>272 (97.85)</td>
<td>128 (100)</td>
<td>0.163 (0.0091 to 2.9180)</td>
<td>0.217</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Case</th>
<th>Control</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (%)</td>
<td>284 (51.07)</td>
<td>128 (50)</td>
<td>1.044 (0.7765 to 1.4039)</td>
<td>0.775</td>
</tr>
<tr>
<td>G (%)</td>
<td>272 (48.93)</td>
<td>128 (50)</td>
<td>0.957 (0.7123 to 1.2878)</td>
<td>0.775</td>
</tr>
</tbody>
</table>

Gathered information

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (95% CI)</td>
<td>56 (52.02–59.98)</td>
<td>37.19 (35.30–39.08)</td>
</tr>
<tr>
<td>Median</td>
<td>55</td>
<td>37.5</td>
</tr>
<tr>
<td>Min–Max</td>
<td>48–64</td>
<td>18–61</td>
</tr>
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</table>

<table>
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<tr>
<th>Height (cm)</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (95% CI)</td>
<td>157.56 (154.95–160.17)</td>
<td>172.31 (170.77–173.86)</td>
</tr>
<tr>
<td>Median</td>
<td>156.00</td>
<td>174.00</td>
</tr>
<tr>
<td>Min–Max</td>
<td>154–162</td>
<td>153–193</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weight (kg)</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (95% CI)</td>
<td>59 (54.25–63.75)</td>
<td>79.05 (76.42–81.69)</td>
</tr>
<tr>
<td>Median</td>
<td>58.00</td>
<td>81.50</td>
</tr>
<tr>
<td>Min–Max</td>
<td>50–69</td>
<td>49–113</td>
</tr>
</tbody>
</table>

Discussion

Breast cancer has numerous numbers of molecular
markers that can be targeted by specific drugs [26, 27].
Alteration in genotype may affect the response of pa-
tient to chemotherapy and may show a wide range of
resistance [28]. Genetic variations in the PI3K–PTEN–
AKT–mTOR pathway affect clinical outcomes in pa-
tients treated with certain types of drugs [17, 29]. The
frequency, breast cancer subtype specificity, and signal-
ing effects of PIK3CA, AKT, and PTEN mutations in
human breast tumors and breast cancer cell lines have
been studied [30]. It has been shown that PIK3CA
pathway aberrations are common in breast cancer [30].
PIK3CA mutations are reported to be presented in ap-
proximately 25% of breast cancer cases, especially in the
estrogen-receptor positive (ER+) and human epider-
mal growth factor receptor 2 (HER2) overexpressing
(HER2+) subtypes [31, 32]; however, the frequency of
PIK3CA mutations in hormone dependent breast can-
cer cell lines was much higher, 39% [30]. This breast
cancer subtype specificity showed that PIK3CA muta-
tions and other PI3K pathway aberrations may play a
distinct role in the pathogenesis and cell proliferation of
the disease [32]. Her2 amplification and PIK3CA mu-
tations often coexist in breast cancer. HER2+/PIK3CA
tumors have also shown drug resistance [33, 34]. It has
been clearly reported that 8% of 12 breast cancers and
4% of 24 lung cancers were associated with PIK3CA so-
matic mutations [12, 35]. 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 [10, 31]. These mutations have
been reported to activate AKT and downstream signal-
ing in model systems [14].
To find out the effects of rs7640662 SNP as a common single nucleotide polymorphism along PIK3CA gene on the risk of breast cancer, 278 cases were analyzed among which 6 (2.15%) cases carrying CC genotype had the higher correlation with the breast cancer compared with CG genotype (97.85%). The P values for CC genotype and CG genotype were 0.094 and 0.217, respectively; however, none of them were associated with the breast cancer (P < 0.05). By reviewing the previous studies, most of abnormalities in PIK3CA signaling function were associated with the mutations mostly occurred in exons and active sites of the enzyme [10, 12, 31, 35]. In the present study, although rs7640662 SNP which is located in intron lack association with breast cancer, PIK3CA intron mutations may play a role in post-transcriptional regulation of exon activity of this crucial enzyme [36]. In addition, this mutation (intron variant) may have important implications for the predicting and diagnostic purposes among families with some cases of breast cancer in their pedigree which needs further research [37].

On the other hand, based on the references released by HapMap project, populations from China and Japan have been reported as Asian populations both of which have 100% CC genotype in rs7640662 [38–40], while for European populations, the frequencies of rs7640662 genotypes were 73.3%, 25%, and 1.7% for CC, GC, and GG, respectively [29]. The frequencies of alleles for European populations were 85.8% and 14.2% for C and G alleles, respectively [29, 38]. In contrast, the present study done in a Persian population showed that the frequencies for both alleles are almost equal. In control samples, we observed frequency of 51.07% and 48.93% for C and G alleles, respectively; however, cancer cases had 50% of frequency for both alleles. GC is the common genotype in Iranian population, and the frequencies observed for GC genotype were 97.85% and 100% in control and case samples, respectively. This shows a strong difference in the Persian population compared with others Asian populations. This study is the first report on the rs7640662 in Iranian population. It is strongly suggested that further research is needed based on larger sample size and in different ethnic population to realize the impact of rs7640662 SNP on breast cancer as the second most frequent kind of cancer. This may cause regional-based drug discovery which leads to localized drug designing to have the best actions of drugs for different ethnic populations based on SNP genotyping.

**Conclusion**

By analyzing the single nucleotide polymorphisms (SNPs) as unique features of genome within a single population, the allelic distribution within a certain population can be concluded for a certain kind of cancer (e.g., breast cancer). Coexistence of single nucleotide polymorphism is also a phenomenon in developing cancer which needs to be evaluated for rs7640662 SNP. However, it eventually results in predicting breast cancer and may realize how effective the mutations are in the mentioned SNP to promote and/or cure of breast cancer.

* * *

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**Authors’ contribution:** AM participated in practical works and writing of the first draft of the manuscript; MHS participated in the follow-up of patients, blood sampling and practical works; ZA participated in acquisition of data and statistical analysis; PJK supervised and designed the study, and wrote and revised the final draft of the manuscript. All authors had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**Conflict of interest:** The authors declare that they have no conflict of interest.

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