RESEARCH ARTICLE

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Evaluation of *PIK3CA, AKT1,* and *mTOR* Genes Polymorphisms in a Sample of Iranian Women with Breast Cancer Compared with Healthy Women

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Abstract

Objective: Breast cancer (BC) is the most common cancer among women globally, with Iranian women showing an earlier onset compared to those in Western countries. Genetic variations in the *PI3K/AKT/mTOR* signaling pathway have been implicated in cancer susceptibility, but limited studies exist on the association of these polymorphisms with BC risk in the Iranian population. This study aimed to investigate the associations of specific polymorphisms in the *AKT1*, *PIK3CA*, and *mTOR* genes with BC susceptibility in an Iranian population. **Methods:** A case-control study was conducted with 222 confirmed BC patients and 230 cancer-free controls. Genotyping of *AKT1* rs1130214 and rs1130233, *PIK3CA* rs6443624, and *mTOR* rs1883965 polymorphisms was performed using PCR-RFLP methods. Statistical analysis was carried out using logistic regression to evaluate the associations between genotypes and BC risk. **Results:** The *AKT1* rs1130214 polymorphism was associated with a significantly reduced BC risk (codominant model: OR=0.44, 95% CI=0.30-0.65, p<0.001; allelic model: OR=0.52, 95% CI=0.38-0.72, p<0.001). Conversely, *AKT1* rs1130233, *PIK3CA* rs6443624, and *mTOR* rs1883965 polymorphisms were linked to an increased BC risk in various inheritance models. For instance, the *mTOR* rs1883965 G>A variant showed a significantly higher risk (dominant model: OR=2.75, 95% CI=1.84-4.12, p<0.001). **Conclusion:** This study indicates that polymorphisms in *AKT1*, *PIK3CA*, and *mTOR* genes are associated with altered BC risk among Iranian women. These findings propose that genetic variants in the *PI3K/AKT/mTOR* pathway could serve as potential biomarkers for BC susceptibility. Further research with larger cohorts and diverse populations is necessary to confirm these associations.

Keywords: Breast cancer, genetic polymorphisms, *AKT1*, *mTOR*, cancer susceptibility

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Introduction

Breast cancer (BC) is the the most prevalent malignancy among women, with more than 1 in 10 new cancer diagnoses annually [1]. In the entire world, it is the second most typical reason for a woman's death from cancer [2]. Notably, breast cancer affects Iranian women nearly ten years earlier than their counterparts in Western countries, which highlights the importance of research on BC in the Iranian population [3].

Genetic variations associated with a higher risk of BC include mutations in the *BRCA1* and *BRCA2 (BRCA1/2)* genes [4]. Clinically, breast cancer is regarded as a heterogeneous disease; while all have similar signs and symptoms, they differ in terms of molecular expression, response to conventional treatments, and prognosis. One conventional classification that is useful in clinical settings is based on the expression level of the progesterone receptor (PR), estrogen receptor (ER), and HER2 (ErbB-2) [5]. A primary characteristic of the onset and advancement of cancer is a change in metabolism toward a greater and quicker rate of energy generation to meet the energy needs of highly proliferating cancer cells [6].

Numerous fundamental physiological processes, including survival, cell growth, motility, metabolism and angiogenesis, are regulated by the phosphatidylinositol 3-kinase (*PI3K*)/AKT/*mTOR* pathway [6, 7]. The *PI3K/AKT/mTOR* pathway is hyperactivated, which causes a significant disruption of normal cellular activities and may contribute to the development of several types

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of cancer in humans [8].

Single nucleotide polymorphisms (SNPs) are the causative factors for differences in phenotypes within populations. SNPs may be found in several areas of the human genome, including promoters, exons, introns, as well as 5′- and 3′ UTRs. Hence, even little modifications in these components might impact gene expression and potentially heighten or diminish susceptibility to certain ailments [9]. Various studies revealed associations between SNPs and different kind of cancers such as breast cancer [10].

The *mTOR* gene, also known as the mammalian target of rapamycin, is located on chromosome 1 (1p36.22) [6]. *mTOR* is a serine/threonine kinase comprising two distinct protein complexes: TORC1, which is sensitive to rapamycin and nutrients, and TORC2, which is responsive to growth factors but remains insensitive to both nutrients and rapamycin. TORC1 plays a pivotal role in promoting cell division and cell cycle progression in response to various stimuli, including amino acids, oxygen levels, stress, energy demands, and growth hormones. TORC2 is activated by growth factors and has a role in controlling cell survival, metabolism, and the cytoskeleton [7]. Overall, the activation of *mTOR* results in enhanced production of several proteins. These include various genes linked to the development of multiple cancers, such as cyclin D1, which enables the passage of cells through the cell cycle, and HIF, which stimulates the production of pro-angiogenic growth factors like VEGF [11].

The *AKT1* gene has been assigned a location on human chromosome 14 (14q32) [6]. AKT, also known as Protein kinase B (PKB), is a serine/threonine kinase that falls under the AGC group, which includes protein kinase A (PKA), protein kinase G (PKG), and protein kinase C (PKC). AKT is crucial in various biological processes such as growth, cell proliferation, metabolism, angiogenesis, protein synthesis, migration, and anti-apoptotic ability [12]. The AKT is a proto-oncogene, and it exists in three different forms, or isoforms, which are named *AKT1*, AKT2, and AKT3. It serves as a target that is affected by the *PI3K* and significantly impacts the survival of cancer cells, their entry into the cell cycle, and their metabolism of glucose [13].

PI3K consists of two distinct subunits, p85 and p110, which regulate its activity and catalytic function. *PI3K* intracellularly mediates different processes like promoting cell transformation, proliferation, tumor initiation, and resistance to apoptosis. The action is triggered by external growth agents and hormones [14]. Approximately 30 to 40 percent of cancer patients have activating mutations in the *PIK3CA* gene, which cause the alpha isoform (p110α) of the phosphatidylinositol 3-kinase (*PI3K*) to become excessively active [15]. *PI3K* activation leads to the production of the second messenger, phosphatidylinositol 3,4,5 trisphosphate (PIP3), from phosphatidylinositol 4,5 bisphosphate (PIP2). The binding of a growth factor to an active receptor tyrosine kinase (RTK) triggers the activation of *PI3K*, which then produces PIP3. This process leads to the activation of many downstream pathways that control numerous cellular processes, including those related to growth of tumors and progression [16].

There is little evidence available about how the AKT/*mTOR*/*PI3K* polymorphism affects an Iranian person's chance of acquiring cancer. In order to investigate any potential links between *AKT1*, *PIK3CA*, and *mTOR* SNPs and breast cancer susceptibility in the Iranian population, case-control research was created.

Materials and Methods

Study Population

This case-control study was conducted on a population comprising 222 confirmed breast cancer patients and 230 cancer-free individuals as the control group. The recruitment protocol received approval from the Ethics Committee of Zahedan University of Medical Sciences (IR.ZAUMS.REC.1402.370). Informed consent was obtained from all participants, including both patients and healthy controls.

Genotyping

Genotyping of the variants was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The specific primer sequences used for this analysis are provided in Table 1.

The determination of all variants was carried out using a reaction mixture containing 1 µL of genomic DNA (~100 ng/µL), 1 µL of each forward and reverse primer (10 µM), 10 µL of 2X master mix (SinaClon, Iran), and 7 µL of ddH2O in a 0.2 mL PCR reaction tube. The PCR protocol included an initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at the appropriate temperature (Table 1) for 30 seconds, and extension at 72 °C for 30 seconds, with a final extension step at 72

Table 1. The Primers Used for Detection of *AKT, PI3K,* and *mTOR* Polymorphisms

Polymorphism	Primer sequence (5'à3')	Annealing (°C)	Restriction Enzyme	Fragment (bp)
AKT1 rs1130214	F: CTGCTGTGGGGTGACTTGTTC	64	XcmI	A allele: 456
	R: AGGTAGTCCAGGGCTGACACA			C allele: 289+167
AKT1 rs1130233	F: CTGCTGTGGGGTGACTTGTTC	68	HpycH4IV	G allele: 272
	R: AGGTAGTCCAGGGCTGACACA			A allele: 208+64
PIK3CA rs6443624	F: TAAGATGTGCAGAGTTCGTTGTATG	53	AluI	A allele: 355
	R: TTGCCTTTGTAAATATGCTCCATAATC			C allele: 262+93
mTOR rs1883965	F: ATGTACCCCAACGATGTAACCA	61	BfaI	G allele: 412
	R: CGGCGTAATGTCCAGACCCA			A allele: 310+102

°C for 10 minutes. Subsequently, 10 µL of the PCR product was digested with a suitable restriction enzyme (Table 1) according to the manufacturer's instructions. The digested products were resolved by electrophoresis on an agarose gel containing 0.5 µg/mL ethidium bromide, visualized under a UV transilluminator, and documented photographically (Figures 1–4). For genotyping quality control, approximately 20% of the samples were randomly selected for blind regenotyping, achieving a reproducibility rate of 100%.

Statistical analysis

All statistical analyses were done using statistical package SPSS 22 software. The categorical and continuous variables were examined using $\chi 2$ and t-test, respectively. The association between genotypes and BC were evaluated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from non-conditional logistic regression analyses. The p-values less than 0.05 were considered as statistically significant.

In silico analysis

For in silico analysis, we utilized two tools, RNAsnp [17] and SpliceAid [18], which are designed to investigate single nucleotide changes on the secondary structure of RNA and the binding of regulatory proteins to RNA, respectively.

Results

Demographics of Study Population

The study group consisted of 222 breast cancer patients (age 48.52 ± 10.77 years) and 230 cancer free subjects as control (49.03 ± 12.09 years). No significant difference was observed between the groups regarding age (P=0.634).

Genotype and Allele Frequencies

Table 2 summarizes the genotype and allele frequencies of each polymorphism evaluated in breast cancer patients and the control group.

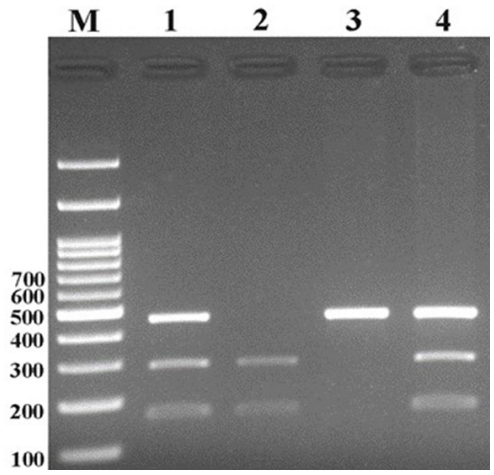

Figure 1. Electrophoresis Pattern of *AKT1* rs1130214 polymorphism. M: DNA marker, lanes 1,4: CA; lane2: CC and lane 3: AA genotypes

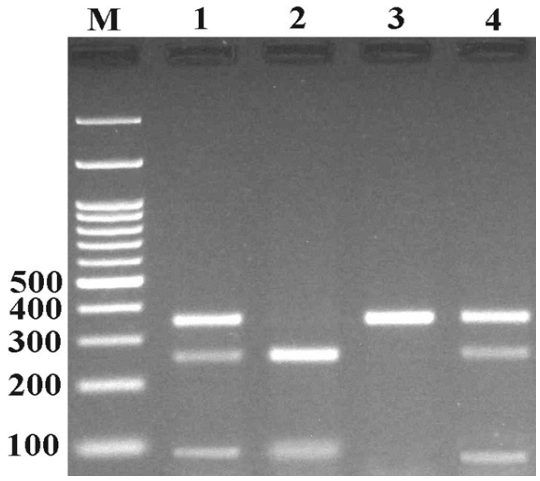

Figure 3. Electrophoresis Pattern of *PIK3CA* rs6443624, M: DNA marker, lanes 1,4:CA; lane 2:CC and lane 3: AA genotypes

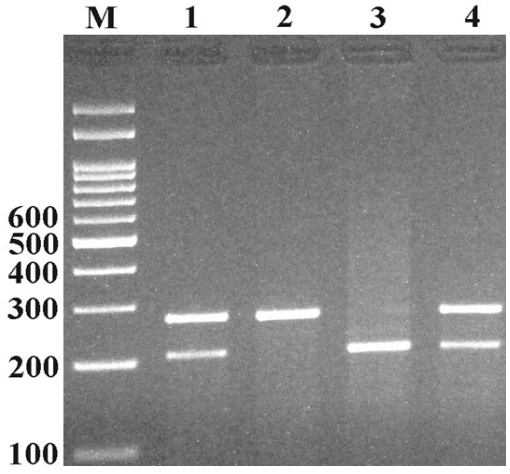

Figure 2. Electrophoresis Pattern of *AKT1* rs1130233, M: DNA marker, lanes 1,4:CA; lane 2:CC and lane 3: AA genotypes

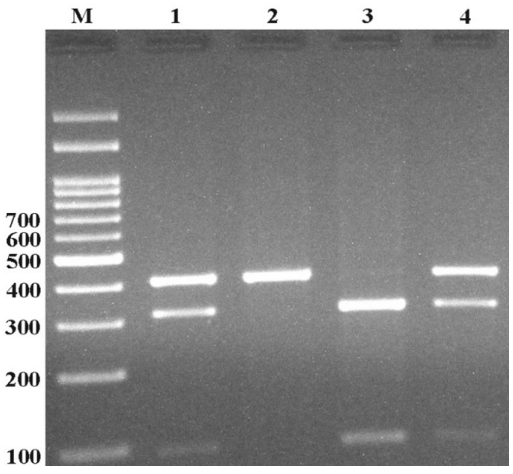

Figure 4. Electrophoresis Pattern of *mTOR* rs1883965 M: DNA marker, lanes 1,4:GA; lane 2:GG and lane 3: AA genotypes

Table 2. The Association of *AKT1, PIK3CA* and *mTOR* Polymorphisms and Breast Cancer Risk

Polymorphism	Model	Genotype	Control n (%)	Case n (%)	OR (95% CI)	P-value
AKT1 rs1130214	Codominant	C/C	109 (47.4)	150 (67.6)	Ref	–
		C/A	106 (46.1)	64 (28.8)	0.44 (0.30–0.65)	<0.001
		A/A	15 (6.5)	8 (3.6)	0.39 (0.16–0.95)	0.037
	Dominant	C/C	109 (47.4)	150 (67.6)	Ref	–
		C/A + A/A	121 (52.6)	72 (32.4)	0.43 (0.30–0.63)	<0.001
	Recessive	C/C + C/A	215 (93.5)	214 (96.4)	Ref	–
		A/A	15 (6.5)	8 (3.6)	0.54 (0.22–1.30)	0.158
	Alleles	C	324 (70.4)	364 (82.0)	Ref	–
		A	136 (29.6)	80 (18.0)	0.52 (0.38–0.72)	<0.001
AKT1 rs1130233	Codominant	AA	93 (40.4)	69 (31.1)	Ref	–
		AG	116 (50.5)	117 (52.7)	1.36 (0.91–2.04)	0.136
		GG	21 (9.1)	36 (16.2)	2.31 (1.24–4.30)	0.008
	Dominant	AA	93 (40.4)	69 (31.1)	Ref	–
		AG + GG	137 (59.6)	153 (68.9)	1.51 (1.02–2.22)	0.038
	Recessive	AA + AG	209 (90.9)	186 (83.8)	Ref	–
		GG	21 (9.1)	36 (16.2)	1.93 (1.09–3.42)	0.023
	Alleles	A	302 (65.7)	255 (57.4)	Ref	–
		G	158 (34.3)	189 (42.6)	1.42 (1.08–1.85)	0.011
PIK3CA rs6443624	Codominant	CC	104 (45.2)	59 (26.5)	Ref	–
		CA	95 (41.3)	122 (55.0)	2.26 (1.49–3.44)	<0.001
		AA	31 (13.5)	41 (18.5)	2.33 (1.33–4.10)	0.003
	Dominant	CC	104 (45.2)	59 (26.5)	Ref	–
		CA + AA	126 (54.8)	163 (73.5)	2.28 (1.54–3.39)	<0.001
	Recessive	CC + CA	199 (86.5)	181 (81.5)	Ref	–
		AA	31 (13.5)	41 (18.5)	1.45 (0.88–2.42)	0.147
	Alleles	C	303 (65.9)	240 (54.1)	Ref	–
		A	157 (34.1)	204 (45.9)	1.64 (1.25–2.15)	<0.001
mTOR rs1883965	Codominant	GG	175 (76.1)	119 (53.6)	Ref	–
		GA	48 (20.9)	92 (41.4)	2.82 (1.85–4.29)	<0.001
		AA	7 (3.0)	11 (5.0)	2.31 (0.87–6.13)	0.092
	Dominant	GG	175 (76.1)	119 (53.6)	Ref	–
		GA + AA	55 (23.9)	103 (46.4)	2.75 (1.84–4.12)	<0.001
	Recessive	GG + GA	223 (97.0)	211 (95.0)	Ref	–
		AA	7 (3.0)	11 (5.0)	1.66 (0.63–4.36)	0.299
	Alleles	G	398 (86.5)	330 (74.3)	Ref	–
		A	62 (13.5)	114 (25.7)	2.22 (1.58–3.12)	<0.001

Association of AKT1, PIK3CA, and mTOR Polymorphisms with Breast Cancer Risk

The analysis indicates significant associations between specific gene polymorphisms in the *AKT1*, *PIK3CA*, and *mTOR* genes and breast cancer risk in the Iranian population. The findings depicted that the *AKT1* rs1130214 SNP notably decreased BC risk in multiple inheritance models. In the codominant model, the CA genotype compared to CC showed significantly decreased odds of breast cancer (OR=0.44, 95% CI=0.30–0.65, p<0.001), as did the AA genotype (OR=0.39, 95% CI=0.16–0.95, p=0.037). The dominant model also showed reduced odds (CA+AA vs. CC; OR=0.43, 95% CI=0.30–0.63, p<0.001), as did the allelic model (A vs. C; OR=0.52, 95% CI=0.38–0.72, p<0.001).

Conversely, the *AKT1* rs1130233 polymorphism notably raised the risk of breast cancer. In the codominant model, the GG genotype compared to AA was correlated with increased odds of breast cancer (OR=2.31, 95% CI=1.24–4.30, p=0.008). Significant associations were also observed in the dominant model (AG+GG vs. AA; OR=1.51, 95% CI=1.02–2.22, p=0.038), the recessive model (GG vs. AA+AG; OR=1.93, 95% CI=1.09–3.42, p=0.023), and the allelic model (G vs. A; OR=1.42, 95% CI=1.08–1.85, p=0.011).

The *PIK3CA* rs6443624 variant was associated with a higher breast cancer risk. In the codominant model, the CA genotype (OR=2.26, 95% CI=1.49–3.44, p<0.001)

and the AA genotype (OR=2.33, 95% CI=1.33–4.10, p=0.003) both showed increased risk compared to the CC genotype. Similar associations were observed in the dominant model (CA+AA vs. CC; OR=2.28, 95% CI=1.54–3.39, p<0.001) and the allelic model (A vs. C; OR=1.64, 95% CI=1.25–2.15, p<0.001).

The *mTOR* rs1883965 variant was also linked to an increased breast cancer risk. In the codominant model, the GA genotype compared to GG showed a significant increase in BC risk (OR=2.82, 95% CI=1.85–4.29, p<0.001). The dominant model (GA+AA vs. GG; OR=2.75, 95% CI=1.84–4.12, p<0.001) and allelic model (A vs. G; OR=2.22, 95% CI=1.58–3.12, p<0.001) also revealed similar risk elevations.

In silico results

The output obtained from the SpliceAid2 tool indicated that the nucleotide change in the rs1883965 polymorphism created a new binding site for the hnRNP DL protein in the mutant state. In contrast, the wild state of this sequence has binding sites for the SRP40 and SF2/ASF proteins (Figure 5). For the rs1130214 polymorphism, SpliceAid2 revealed that in the mutant state, there were five binding sites for hnRNP H1, hnRNP H2, hnRNP H3, hnRNP H4, and KSRP proteins. The nucleotide change from G to T removed these proteins identification sites, creating only one site for the MBNL 1 protein. The results from RNAsnp for the rs1130214 polymorphism indicated that the nucleotide change leads to a significant structural change in the RNA's secondary structure. Unmodified results are not displayed.

Discussion

Breast cancer (BC) is the most common form of cancer and the primary reason for cancer-related deaths among women globally. Our study, conducted in an Iranian population, emphasizes the observation that BC occurs at an earlier age in Iran compared to Western populations, with the average age of diagnosis ranging from 47.1 to 48.8 years, which is at least 10 years sooner than in Western nations [1].

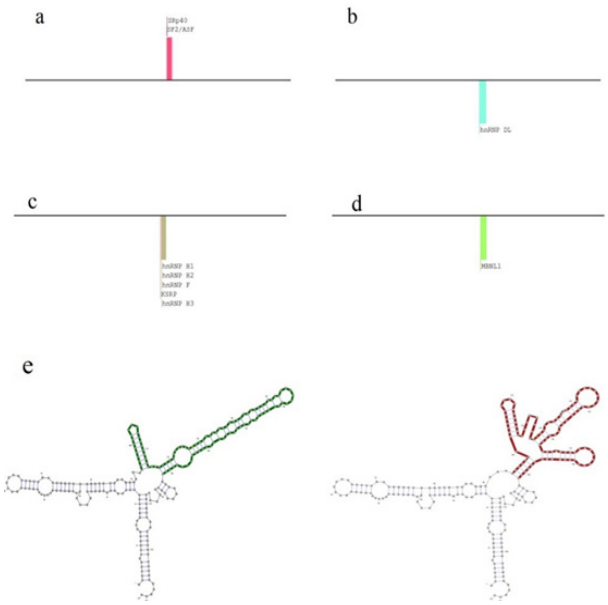

Figure 5. Changes in the Binding of Regulatory Proteins to Wild-Type and Mutant Alleles of the *mTOR* and *AKT1* genes at the polymorphic variants rs1883965(a,b) and rs1130214(c,d). Significant change in RNA structure due to nucleotide change from G to T in AKT1 gene polymorphism rs1130214 (e).

In this study, we examined the association between breast cancer susceptibility and polymorphisms in key genes *AKT1*, *PIK3CA*, and *mTOR* within the *PI3K/AKT/mTOR* pathway. This pathway regulates fundamental cellular processes, including cell growth, proliferation, migration, and survival. The *PI3K/AKT/mTOR* pathway is often hyperactivated in cancers, potentially due to genetic polymorphisms that disrupt cellular homeostasis.

The dysregulation of the *AKT1* gene, such as through genetic variants, has been previously associated to a large number of diseases, including cancer (particularly breast cancer), Alzheimer, Huntington, diabetes mellitus, cardiovascular and neurological disorders, and other metabolic disorders [19]. Our results identified that the *AKT1* rs1130214 polymorphism significantly reduced BC risk, while *AKT1* rs1130233 was associated with an increased BC risk. Specifically, the rs1130214 SNP exhibited a protective effect against BC, potentially by altering *AKT1* signaling and reducing its influence on cell proliferation and survival pathways. The protective association of the *AKT1* rs1130214 variant aligns with studies by Ilozumba et al., who observed a reduced BC risk among specific populations with this variant [20]. In addition, Li et al., reported the correlation of *AKT1* rs1130214 and decreased risk of head and neck squamous cell carcinoma in recessive and dominant models [21]. Conversely, the rs1130233 variant's risk-enhancing effect has been similarly noted in gastric [22] and colorectal [23] cancers, where the AA and GA genotypes elevated cancer susceptibility. These associations show the pleiotropic effects of *AKT1* polymorphisms, where variations may predispose individuals to different cancers depending on environmental and genetic backgrounds.

The *mTOR* gene plays a vital role in the *PI3K/AKT/mTOR* signaling pathway, and when it is not properly regulated, it is linked to the development and advancement of many types of malignancies [24]. Our result showed a significant association between *mTOR* rs1883965 variant and increased breast cancer risk, aligning with findings from studies in various cancers reported by Min et al. and Zining et al. According to Zining et al, variant rs1883965, which is likely a transcription factor binding site of *mTOR*, was found to be related with esophageal carcinoma, hepatocellular carcinoma, and gastric cancer [25]. In 2022, Min and colleagues published a report exhibiting the significant association of rs2295080 T/G polymorphism with cancer susceptibility, particularly for malignancies affecting the urinary system, breast, and blood. Moreover, the rs1883965 G/A variant was found to be linked to an increased risk of developing cancer, particularly stomach cancer [26]. In the study conducted by Zhu et al., it was shown that those who had the A-allele of the rs1883965 G/A SNP had a 1.12-fold increased risk of developing cancer compared to those who

carried the G-allele. This observation was made based on the analysis of pooled data. Furthermore, when analyzing the data based on different types of cancer, it was shown that the rs1883965 G/A polymorphism was associated with a higher risk of developing digestive cancer. This link was observed while comparing the presence of different alleles, comparing individuals with one copy of the variant allele to those with two copies, and using a model where the presence of the variant allele dominates [27, 28].

The *PIK3CA* rs6443624 variant was another significant factor in increasing breast cancer risk within our sample. Agell et al. found no association between the disease and the variations of *PIK3CA* (rs6443624, rs7641889, rs7641983, rs2699905, rs7651265, rs2677760, rs7640662, rs1607237, rs3729692, rs6786049). There is a proposal suggesting that the activation of the *PI3K* signaling pathway in prostate cancer (PC) is caused by the over-expression of *PIK3CA* mRNA [29]. According to Wang et al., the *PIK3CA* rs9838411 and rs6443624 variations were reported to either somewhat or dramatically reduce the incidence of endometrial cancer in a dominant model [30]. In 2015, bodnar and colleagues performed a study on the *PI3K*CA rs6443624 gene and discovered a substantial correlation between this gene and the survival rates of patients with renal cell carcinoma (RCC) who were treated with everolimus. An analysis of the relative risk using a dominant model revealed a two-fold higher likelihood of mortality in individuals with genotype AC or AA compared to those with the variation CC [31]. Given that *PI3K* activation is an upstream driver of the AKT/*mTOR* pathway, individuals with this variant may experience compounded signaling effects that accelerate cancer progression.

In conclusion, genetic variations in *AKT1*/*mTOR*/*PIK3CA* genes can be used as prognostic indicators for breast cancer and risk of developing BC in a sample of Iranian population. Confirming these results in larger sample sizes and a wider range of racial and ethnic groups would provide more evidence that *PI3K*/*AKT1*/*mTOR* pathway variants play a role in the development of breast cancer. Additionally, future studies should explore the functional mechanisms of these polymorphisms within the *PI3K/AKT/mTOR* pathway to better understand how they modulate cancer risk at the molecular level. Expanding our knowledge of these genetic factors could support the development of targeted therapies, advancing personalized medicine for breast cancer patients.

Author Contribution Statement

AN, MT and SMH recruited the subjects, collected clinical data. RM prepared materials, performed experiments, and drafted the manuscript. GB designed the study, analyzed the data, interpreted data, and approved the final version and proofread the manuscript. All authors approved the final manuscript.

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Ethical Approval

The project was approved by the Local Ethics Committee of Zahedan University of Medical Sciences (IR.ZAUMS.REC.1402.370), and written informed consent was obtained from all participants.

Data availability

The datasets generated during and/or analyzed dur¬ing the current study are available from the corresponding author on reasonable request.

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Conflicting Interest

The authors declare that they have no conflicting interests.

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