Original Article

Evaluation of AURKA rs2273535 and CDKN1B rs34330 Polymorphisms Association with the Risk of Breast Cancer

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Abstract

Background & Objective: Breast cancer consists of a heterogeneous group of tumours with different prognosis and is the most common cause of cancer-related mortality among women. In this study, our goal was to evaluate the association of AURKA rs2273535 and CDKN1B rs34330 polymorphisms with risk of female breast cancer in southwest part of Iran.
Materials & Methods: This case-control study was done on 50 women with breast cancer and 50 healthy women without any symptoms or family history of breast cancer as the case and control group, respectively. Restriction fragment length polymorphism (RFLP- PCR) technique was designed to determine the AURKA rs2273535 and CDKN1B rs34330 gene polymorphisms. Afterwards, statistical analysis was done by means of SPSS (version 17).
Results: In current research, our finding showed that rs2273535 T allele increased the susceptibility of breast cancer (OR:2.58, 95%CI:1.5-31.09, P=0.006). Moreover, T allele in dominant phase could raise the risk of the breast cancer (OR:3.01, 65%CI:1.31-6.92, P=0.009). However, the other polymorphism, CDKN1B rs34330, revealed no associations with increased risk of breast cancer.
Conclusion: These findings suggest that AURKA rs2273535 may influence individual's susceptibility to breast cancer. But we found no associations regarding CDKN1B rs34330 polymorphism and this type of cancer.

Keywords: Breast cancer, AURKA, CDKN1B, rs2273535, rs34330, RFLP

Introduction

Breast cancer has been ranked as the most frequent cancer and the major cause of female cancer-related mortality globally, derived from the epithelium of the mammary gland (1). Based on the data from the GLOBOCAN 2012, generated by the International Agency for Research on Cancer (IARC), breast cancer incidence could be as high as 1,676,600 new cases annually (2). Gene expression analysis classifies this type of cancer into various molecular subtypes which is currently considered a heterogeneous disease. These molecular subtypes are composed of basal cell-like, Her-2/neu, luminal A, and luminal B (3). Furthermore, breast cancer is a multi-factorial disease which includes several items including age,

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level of estrogen, lifestyle and reproductive factors. A pile of genetic factors would be the cause of this cancer as well (4). The heterogeneity of the cancer exerts a serious challenge in its diagnosis and treatment. Clinical presentation, disease aggressiveness, and treatment response could vary from patient to patient (5). This challenge would be due to the lack of complete vision of the biologic heterogeneity in relation to molecular alterations, treatment sensitivity, and cellular composition. Tumourigenesis is originated from the alternation in the genetic material involved in the expression of proteins in cell cycle progression (6).

The promotion of the cell cycle happens by activation of cyclin-dependent kinases, positively and negatively regulated by cyclins and cyclin-dependent kinase inhibitors, respectively (7). P27 protein inhibiting the enzymatic function of cyclin-CDK complexes is encoded by the CDKN1B gene. This protein is indispensable for the progression from the G1 to the S phase of the cell cycle (8). P27 inactivation or its proteolysis acceleration is reported in several human cancers, including breast cancer (9). Furthermore, loss of CDKN1B expression which is ubiquitous in breast cancer has been significantly related with high tumour grade and poor prognosis (8).

Aurora kinase A (AURKA) activation is crucially significant in the control of mitosis progression, centrosome maturation/separation and mitotic spindle function (10). At the beginning of S phase, AURKA localizes to the duplicated centrosomes, then, during mitosis it shifts to the bipolar spindle microtubules, and at the final stage of mitosis, moves to perinuclear materials of the daughter cells (11). Aurora kinases deregulation results in mitotic spindle checkpoints defect causing abnormal spindle assembly (12). AURKA overexpression which has been reported to be associated with genetic instability and tumourigenesis on account of disrupting the proper assembly of the mitotic checkpoint complex occurs in several cancers such as breast cancer (13, 14). Studies demonstrated single-nucleotide polymorphisms (SNPs) in AURKA or AURKB have great impact on the risk of breast cancer (15). In the current study, we aimed to investigate the association of AURKA rs2273535 and CDKN1B rs34330 gene polymorphisms with susceptibility of breast cancer in the southwest of Iran.

Materials &Methods

Subjects

This case-control study was conducted on 100 women who were referred to Narges Pathology Laboratory from March 2015 to February 2017. Patients consisted of subjects who had surgically and histologically diagnosed with breast cancer and were aged-matched with healthy women blood donors. Of these individuals, 50 (50%) were affected and 50 (50%) were unaffected by breast cancer without history of any type of cancers used as case and control groups, respectively. Both groups were reported to belong to the same ethnic (Caucasian) group. Peripheral blood was collected from the studied participants after obtaining their signed consent. Genomic DNA was extracted from peripheral whole blood specimen using the DNA extraction kit (SinaClone, Iran). This study was conducted after being approved by Marvdasht Islamic Azad university (ethical code: IR.IAU.M.REC.1399.019). The time of getting ethical code was different from sample preparation. Due to COVID-19 outbreak, we had to use pre-prepared samples.

Genotyping

AURKA rs2273535 and CDKN1B rs34330 polymorphisms were genotyped using restriction fragment length polymorphism (RFLP- PCR) technique. The designed primers listed in Table 1 were designed by a software program called primer design. Regarding AURKA rs2273535, we designed a mismatch. This was added to create a cleavage site for the MfeI enzyme. This method has been used to differentiate for polymorphism detection. The SNP of rs2273535 is at position 16 of forward primer but the mismatch is at position 14. It can be due to the fact that, the sequence around the desired region naturally lacks the cut site by restriction enzymes. Fortunately, such

a cutting position can be created with a small change. Therefore, by designing the primer, we simultaneously replicated the desired area and created a restriction site for Restriction Fragment Length Polymorphism (RFLP).

Table 1. the characteristics of primer sequences, PCR products, and restriction enzymes of rs2273535 and rs34330

polymorphisms	primers	PCR products	Restriction enzymes
rs2273535	F: $(A)_{19}$ $(C)_{20}$ GATTCTGACAAGGCAATTGC R: CTAACTATCCTCTGGAGGTACTG	After RFLP: normal allele: 2 fragments: 54 bp and 216 bp Mutant allele: 1 fragment: 270 bp	MfeI
rs34330	F: TCGCCAGTCCATTTGATC R: GGTTAACTCTTCGTGGTCCAC	After RFLP: normal allele: 3 fragments: 64 bp and 153 bp and 203bp (90bp and 292 extra) Mutant allele: 356 bp (292 bp and 64 bp extra)	BstuI

The PCR tests were carried out in the MyCyclerTM thermal cycler system (Bio-Rad, United States). The final mix volume was 25µL, containing 2.5 ml PCR buffer x10, 1µl of primers (10 pmol/mL), 1.5mM of $MgCl_2$, 1mL of dNTP (10mm), 0.2 ml of Taq DNA polymerase, double distilled water and 1 µL template DNA (100 ng/mL).

PCR amplification conditions were as below: an initial denaturation at 94°C for 5 min followed by 30 cycles with the following conditions: 94°C for 45 sec, 53°C (RS34330) or 58°C (RS2273535) for 45 sec and 72°C for 45 sec with a final extension at 72°C for 7 min. To digest PCR products, MfeI and BstuI restriction enzymes were used for genotyping of rs2273535 and Rs34330 polymorphisms, respectively. The products were verified by electrophoresis in 2% agarose gels. It should be noted that MfeI restriction sequence is CAATTG, so the sequence is digested by the enzyme if the normal allele is present. If the mutant allele is seen (CATTTG), the sequence is not recognized by the enzyme. Therefore, we should not expect all sequences to be cut.

Statistical analysis

Statistical analysis was done by means of SPSS for windows version 17 software (SPSS Inc., Chicago, IL). Tests for Hardy–Weinberg equilibrium were performed applying $\chi 2$ test performed in order to make a comparison

between the observed and expected genotype frequencies. To calculate 95% confidence intervals (CI) and odds ratio (OR) of genetic susceptibility to this type of cancer, logistic regression analysis was used. P-values of less than 0.05 were considered significant.

Results

Were all age-matched (±5) and the patients had mean of 48.6±28.11 years old and 34-64 range. Control group participants were in range of 38 to 63 years old and the mean of their ages was 51.02±6.9. There was no significant difference among groups regarding age (P=0.29). The information regarding age is represented in Table 2.

Table 2. characteristics of study group in terms of number, mean, and range

Variables	controls	patients	P
Range of ages (year)	38-63	34-64	
mean (year)	48.6±28.11	51.02±6.9	(P=0.29)
numbers	50	50	

After replicating DNA via PCR, the rs2273535 and Rs34330 PCR products were digested with MfeI and BstuI restriction enzymes, respectively. The electrophoresis pattern of RFLP-PCR for detection of rs2273535 and Rs34330 polymorphisms is shown in Figure 1.

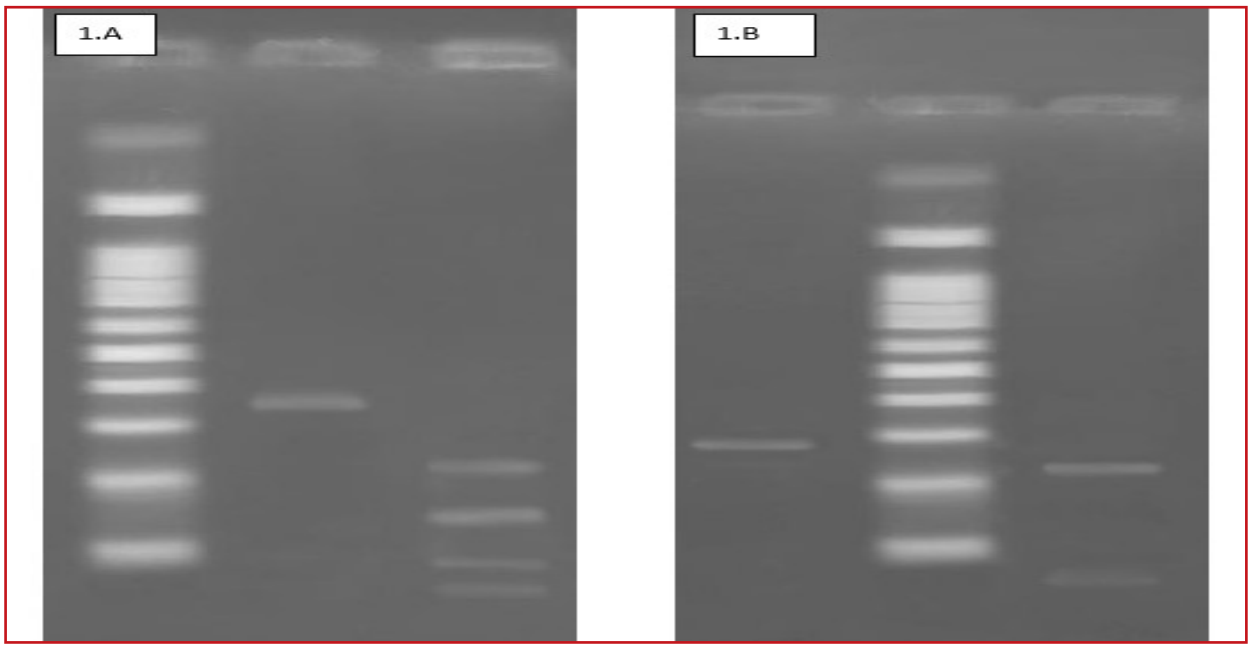

Figure 1. These figures represent the electrophoresis pattern of rs34330 and rs2273535. 1.a: 356 bp before digestion and 64,90,153 and 203 bp for rs34330. 1.b shows 270 bp fragmentation size before digestion and 54, 216 bp after using restriction enzyme for rs2273535

Concerning Rs34330 polymorphism, PCR product size before digestion was 356 base pairs, then, it would be 64, 90, 153, and 203 bp. When it came to the rs2273535 polymorphism, 270 bp was the size of product before using restriction enzyme. Afterwards, it would be 54 and 216 bp.

The genotype frequencies of the control (χ2:19.42, df:2, $p<0.05$) and case (χ2:20.86, df:2, $p<0.05$) groups in case of rs34330 polymorphism showed that they were not in the Hardy–Weinberg equilibrium.

Detailed genotype distributions and the association between rs34330 polymorphism and breast cancer risk are illustrated in Table 3.

Table 3. Association of rs34330 polymorphisms with breast cancer risk

polymorphism			Patients (%)	OR (CI95%)	P*
genotype	TT	8 (16.0%)	3 (6.0)	1	-
	TC	40 (80.0%)	41 (82.0%)	2.53 (0.62-10.26)	0.19
	CC	2 (4.0%)	6 (12.0%)		
	TC+CC	42 (84%)	47 (94%)	2.98 (0.74-11.93)	0.19
Allele	T	56(56%)	47(47%)	1	-
	C	44 (44%)	53 (53%)	1.43 (0.82-2.5)	0.12

*Regression logistic test

The statistical result showed that there was no significant association between the rs34330 polymorphism and susceptibility of breast cancer in Iranian female population.

The result of genotype distribution in two groups revealed that the control group (χ2:0.57, df:2, $p>0.05$) and cases (χ2:0.12, df:2, $p>0.05$) concerning rs2273535 polymorphism were in the Hardy–Weinberg equilibrium.

In current study, the relationship between rs2273535 and breast cancer risk was surveyed and as it was illustrated in Table 4, T allele increased breast cancer risk (OR:2.58, 95%CI:1.5-31.09, P=0.006). In addition to this, T allele in dominant phase would increase the risk of this type of cancer (OR:3.01, 65%CI:1.31-6.92, P=0.009).

Table 4. The association of rs2273535 polymorphism with breast cancer risk

Genotype/Allele	Control (%)	Patient (%)	OR(%95CI)	P	*P
Codominant					
AA	36 (72.0)	23 (46.0)	1	-	-
AT	12 (24.0)	21 (42.0)	2.73 (1.13-6.61)		0.025
TT	2 (4.0)	6 (12.0)	4.69 (0.87-25.28)		0.072
Dominant					
AA	36 (72.0)	23 (46.0)	1		
AA+AT	14 (28.0)	27 (54.0)	3.01(1.31-6.92)		0.009
Recessive					
AA+AT	48 (96.0)	44 (88.0)	1		
TT	2 (4.0)	6 (12.0)	3.27 (0.62-17.0)		0.15
Allele					
A	84 (84.0)	67 (67.0)	1	-	
T	16 (16.0)	33 (33.0)	2.58 (1.31-5.09)		0.006

Discussion

There are a number of safeguards and checkpoints which are necessary for the limited cell cycle are overridden by cancer cells and enable limitless proliferation. The variety of genetic and epigenetic molecular alternations involve in the cancer formation. These items would lead to hyperactivating or inactivating major components of the cell cycle in order to keep aberrant proliferation. Different breast cancer subtypes can be varied in terms of various molecular changes and reliance on the cell cycle and its checkpoints (16). Breast cancer is defined by lack of cell cycle control via either tumor suppressors inactivation or aberrant activation of cyclins and cyclin-dependent kinases (CDKs) (16). Therefore, we performed two experiments on two polymorphisms (AURKA rs2273535 and CDKN1B rs34330) which are engaged in cell cycle regulation and determined their connection with breast cancer susceptibility.

AURKA plays a key role in regulating G2 to M transition during mitosis. The AURKA protein consists of not only a 129-amino acid N-terminal domain facilitating AURKA nuclear-translocation during mitosis, but also a 274-amino acid C-terminal kinase catalytic domain (17). The role of threonine kinase belonging to a family of mitotic kinases is to maintain chromosomalstability through phosphorylation. Any severe defects in AURKA,

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accordingly, mutations can be a case in point, would result in dramatic genomic instability and inducing apoptosis through cell cycle checkpoint surveillance (10). As a result, cancer may be derived from the cells harboring a defective AURKA (14). AURKA rs2273535 T>A being a transversion at position 91 in this gene coding sequence (T-to-A) is also known as F31I or Phe31Ile (18). rs2273535 polymorphism effect entails more than just cellular transformation and chromosomal instability enhancement (19). In addition to this, an obstruction in p53 binding and reduction in the degradation of AURKA by changing the activity of the AURKA box 1 can be caused by this polymorphism (20). Due to dire effect this polymorphism exerts on cancer evolution, we picked it up for its impact on susceptibility of breast cancer in Iranian population. The relationship between rs2273535 and breast cancer risk was evaluated in the current study and based on our finding, T allele increased the risk of breast cancer (OR:2.58, 95%CI:1.5-31.09, P=0.006). Moreover, T allele in dominant phase would escalate the risk of the cancer (OR:3.01, 65%CI:1.31-6.92, P=0.009). In a case-control study recruited by Sun et al, an increased risk of breast cancer susceptibility associated with the Ile/Ile genotype of rs2273535 was identified (21). The similar result has been found in the study of Ruan et al (22). On the other hand, in 2015, Taylor et al reported no association between rs2273535 and breast cancer among population of African American women (23). Although in Die and colleagues' investigation there was no significant relationship with breast cancer and Ile/Ile homozygotes (rs2273535, Phe31Ile), the enhanced risk of this cancer was found in this study in overweight postmenopausal women carrying Ile/Ile homozygotes (24).

The other polymorphism which was investigated by our team was the CDKN1B rs34330 polymorphism. The cyclin-dependent kinase inhibitor 1B (CDKN1B) gene encodes p27 protein. This gene is mapped to chromosome 12p13. Enzymatic activation of cyclin E/CDK2 complexes is deterred by the cell cycle inhibitor protein and this protein promotes cell cycle arrest at G1 phase (8). The rs34330 polymorphism which has been reported to be situated in the 5′-untranslating region of p27 gene might be correlated with the reduced rate of p27 protein production (25) and it would alter p27 transcription (26). Because of rs34330 effect on p27 protein generation, its connection with breast cancer is definitely worth to be studied particularly in different ethnicities. We, as a result, selected this polymorphism. The result of the current study showed that there was no significant association between the rs34330 polymorphism and susceptibility of breast cancer in the population of Iranian women. It might be due to the study size which was relatively small. With considerably more samples, different results might be attained. The same result has been found in the investigation of Mcinerney et al on Irish women (27). However, Canbay's study (6) indicated that the CDKN1B C-79T heterozygote, but not the homozygote, substantially increased the risk of breast cancer. It might be in the light of the fact that this polymorphism leads to protein dysfunction. Because of this, the potential imbalance of the protein structure might be evolved. Moreover, the association between T allele of rs34330 and risk of cancer risk was confirmed in Chinese women (28).

Conclusion

In conclusion, we indicate that in the Iranian population, AURKA rs2273535 gene polymorphisms is associated with breast cancer risk. Unlike that, CDKN1B rs34330 gene polymorphism did not show any significant association with this multifactorial disorder. This could provide the source of information for further studies. Larger studies are needed to confirm these results in various ethnicities.

Conflict of Interests

The authors declare that they have no competing interests.

Acknowledgments

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