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The rs4415084 and rs10941679 Might be Risk Conferring Factors for Breast Cancer among Iranian Women

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ABSTRACT

Breast cancer is a major cause of cancer-associated death among Iranian women with an increasing trend in breast cancer incidence in recent years. However, few data about genetic determinants associated with breast cancer among Iranian females are available. According to the literature, the genetic variants at the non-coding region of 5q12 play a critical role in breast cancer susceptibility. Therefore, this study aims to determine the association of rs4415084 (T>C) and rs10941679 (A>G) with breast cancer risk in an Iranian sample population. Peripheral blood samples, taken from 72 patients and 65 controls, were subjected to DNA extraction and genotyping of the mentioned polymorphism by Tetra-primer amplification refractory mutation system PCR (Tetra-ARMS-PCR) assay. Also, the demographic characteristics of the participants were obtained. The results show that the majority of patients had estrogen-receptor (ER) and progesterone-receptor (PR) positive tumors (75% for either receptor) and 36 % were human epidermal growth factor receptor-2 (HER2) positive. Also, the majority of the tumors were at T2 (59.7 %), N0 (41.6 %), and M0 (86.1 %) grade. Moreover, a considerable association between the rs10941679 polymorphism and breast cancer was noticed (p=0.01) with significantly increased susceptibility of AG genotype. Genotyping of rs4415084 also revealed that TC and CC genotypes significantly increased breast cancer risk. This work revealed that the rs4415084 and rs10941679 at 5q12 might be risk-conferring factors for breast cancer among Iranian women, which could be employed in breast cancer screening and prognosis approaches.

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Introduction

Breast cancer is regarded as the most common cancer in females, worldwide (Qin *et al.*, 2012; Colagar *et al.*, 2015). Over 1.67 million women are annually affected by breast cancer, and significantly higher susceptibility is observed among the relatives of affected women (Mcinerney *et al*., 2009). In recent years, there has been an increasing trend in breast cancer incidence in the Iranian population, reaching from 16.0 to 28.3 per 100000 women, which is the most commonly diagnosed cancer in Iranian females (Mirzaeyan *et al.*, 2020; Salehiniya *et al*., 2018).

Several genetic variants including a variety of rare coding and non-coding susceptibility loci have been associated with breast cancer risk (Michailidou *et al*., 2017). Thus, considering the association of genetic variations at the non-coding regions with breast cancer susceptibility is a novel approach to determine the relationship between genetic characteristics and breast cancer susceptibility (Liang, 2016). In this regard,

several genome-wide association studies (GWAS) on breast cancer were conducted which introduced novel susceptibility loci. Based on these studies, several non-coding variants located at 8q24, 2q35, and 5p12 were introduced (Easton *et al*., 2007; Stacey *et al*., 2007).

According to the literature, the increased breast cancer susceptibility at 5p12 is associated with two single nucleotide polymorphisms (SNPs), including rs4415084 (T>C) and rs10941679 (A>G) (Stacey *et al*., 2008; Ghoussaini *et al*., 2016). Two single nucleotide polymorphisms (SNPs), rs4415084, and rs10941679 on chromosome 5p12 were associated with the risk of breast cancer in a recent genome-wide association study (GWAS) of women with European ancestry. Both SNPs are located in a large high-LD region and the causal variant(s) are still unknown (Ruiz-Narvaez *et al*., 2010). Previous reports associated these SNPs with increased breast cancer risk in different populations, including Caucasians and East Asians; however, the results were contradictory in different ethnic groups. Also, the mentioned SNPs on 5q12 were reported to confer risk for estrogen receptor-positive tumors (Yu *et al*., 2013).

Very few studies aimed to determine the association of rs4415084 and rs10941679 with breast cancer among Iranian women. Owing to the recent increasing trend of breast cancer incidence in Iran, the characterization of genetic determinants associated with breast cancer in Iranian females would be an efficient approach in disease prognosis and screening programs. Thus, the current work aimed to determine the association of two SNPs at 5p12, including rs4415084 and rs10941679 with breast cancer risk in an Iranian female population.

Materials and Methods

Patients

This case-control study was conducted on a total number of 137 women, including 72 breast cancer patients and 65 control individuals. The patients were selected from the women referring to the Hazrat Rasoul medical complex (Tehran, Iran) from January to July 2017. The inclusion criteria for the cases were 1) confirmed breast cancer by histopathological examination of breast biopsies, and 2) without a history of other cancers. Clinical and demographic characteristics of patients, including estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) status, and pathological grades were determined. The healthy controls were selected randomly from the women without a history of any cancer.

3Prior to participation in the study, informed consent was obtained from the subjects. This study was ethically approved by the local ethical committee of Islamic Azad University (Rasht Branch).

Tetra-ARMS-PCR

Peripheral blood samples were collected from each subject in EDTA tubes and transferred immediately to the laboratory, where nuclear DNA extraction was performed using the GeNet BIO Genomic DNA Kit (South Korea) according to the manufacturer's instruction. Tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR) was used for the genotyping of 512q susceptibility loci using a pair of outer primers and two allele-specific primers for each SNP (Table 1). Primer design was performed using the Primer3 software based on the sequences from the database.

Table 1. Primer sequences were used in this study.

Variant	Primers	Sequence (5'→3')	Allele	Length (bp)	Tm (°C)
rs10941679 (A>G)	F- outer	CTGTAAGCAAGTATATCAGTTTGGGGATTT		429	64
	R- outer	AGGGCAATAGAAATTCCATAGAAAACAA			
	F- inner	AGTAAAATGTGGGATGCTTTTTATTGAATG	G	197	64
	R- inner	TGCTTTTTTTATGCTGTGTTCTTTCAAT	A	289	
rs4415084 (C>T)	F- outer	GCTGTTTGTTCTATTTCTTTCTCATCTGTCCCA		654	70
	R- outer	AAAACTGGATCCCAAGAGGGTTTGAGTGTATTA			
	F- inner	TCTAGCCCTGTTGTATTCCTGATGACTTGAGAAC	C	426	70
	R- inner	ATTGACCAGTGCTGTATGTATCACTCCCTTTGA	T	294	

The Tetra-ARMS-PCR was performed in a total volume of 25 µL containing 1µg of template DNA, 1.5/1.5/3.5/2.5 µL of outer and inner primer pairs (Bioneer, South Korea), 1 µL of $MgCl_2$, 0.5 µL of mix dNTPs, 2.5 µL of PCR amplification buffer, 0.3 µL Taq DNA polymerase (CinnaGen, Iran) and 10.7 µL of distilled water (for rs4415084) and a total volume of 25 µL containing 1µg of template DNA, 1/1/1/1 µL of outer and inner primer pairs, 1 µL of $MgCl_2$, 0.5 µL of dNTPs, 3 µL of PCR amplification buffer, 0.5 µL Taq DNA polymerase, and 15 µL of distilled water (for rs10941679). Amplification of target sequences was performed as follows: Initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 64 °C for 30 s and extension at 72 °C for 30 s, and final extension at 72 °C for 5 min in a thermo-cycler instrument (Peqlab, Germany).

Finally, the Tetra-ARMS PCR products were visualized using 1.5% gel electrophoresis and the genotype of each individual was determined. For rs10941679, the presence of 197 or 289 bp represents the G and A alleles, respectively. Also, for rs4415084, the C and T allele-specific bands are represented by the 426 and 294 bp amplicons, respectively.

Statistical analyses

Statistical analyses were performed using SPSS.16 software (SPSS Inc., IL). The frequency of each genotype for rs10941679 and rs4415084 susceptibility loci were determined. Then, the Logistic Regression Model and Chi-square test were used to determine the association between different genotypes and the risk of breast cancer. The p-values of less than 0.05 were considered statistically significant.

Results

Populations' demographic characteristics

Demographic characteristics of breast cancer patients and healthy controls were presented in Table 2. The mean age of the case and control groups was compared and no significant difference was noticed (p= 0.001). The mean age of the patients and healthy controls were 62 and 70, respectively. The majority of patients had ER and PR positive tumors (75% for either receptor) and 36 % were HER2 positive. Also, 26.3 % of patients had $ER^+PR^+HER2^-$ tumors which were the most prevalent category based on hormone receptors of breast tumors. The Patients mainly suffered from breast tumors in either breast (90 %), or the majority of the tumors were at T2 (59.7 %), N0 (41.6 %), and M0 (86.1 %) grade.

Table 2. Demographic characteristics of the studied population.

Characteristics		Patients (n= 72)	Healthy (n= 65)
Mean age		70	62
Age of diagnosis (<40)		13 (18.0 %)	
Family history		29 (40.2 %)	
ER status	Positive	54 (75.0 %)	
	Negative	18 (25.0)	
PR status	Positive	54 (75.0 %)	
	Negative	18 (25.0)	
HER_2	Positive	26 (36.1 %)	
	Negative	46 (63.8 %)	
Hormone pattern	$ER^+PR^+HER_2^+$	18 (13.1 %)	
	$ER^+PR^+HER_2^-$	36 (26.3 %)	
	$ER^-PR^-HER_2^+$	8 (5.8 %)	
	$ER^-PR^-HER_2^-$	10 (7.3 %)	
Histologic grade	1	38 (52.8 %)	
	2	21 (29.2 %)	
	3	13 (18.1 %)	
lymph node involvement status	0	6 (8.3 %)	
	1	30 (41.7 %)	
	2	28 (38.9 %)	
	3	8 (11.1 %)	
Distant metastasis	NO	63 (87.5 %)	
	Yes	9 (12.5 %)	
T stage	1	24 (33.3 %)	
	2	44 (61.1 %)	
	3	4 (5.6 %)	

ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor-2,

Genotypes and allele frequency

Prevalence of rs10941679 and rs4415084 among breast cancer patients, and healthy controls was determined. Evaluation of rs4415084 showed a prevalence of 22.2 % (n=16), 59.7 % (n=43), and 18.1 % (n=13) for CC, CT, and TT genotypes among breast cancer patients, while the control group showed a prevalence of 43.1 % (n=43) for CC, 24.6 % (n=16) for CT, and 9.2 % (n=6) for TT genotypes.

Also, evaluating the rs10941679 among the cases revealed a prevalence of 23.6 % (n=17), 54.2 % (n=39), and 22.2 % (n=16) for AA, AG, and GG genotypes, respectively. In contrast, among the controls the AA, AG, and GG genotypes had a prevalence of 47.7 %, 33.8 %, and 18.5 %, respectively (Table 3).

Table 3. Genotype and allele frequency of rs10941679 and rs4415084 polymorphisms among the studied population

Polymorphisms		Genotype frequency n (%)			Allele frequency n (%)	
		AA	AG	GG	A	G
rs10941679	Cases	17 (23.6)	39 (54.2)	16 (22.2)	73 (50.7)	71 (49.3)
(A>G)	Controls	31 (47.7)	22 (33.8)	12 (18.5)	84 (64.6)	46 (35.4)
		χ^2= 9.058, p= 0.01*			χ^2= 5.411, p= 0.014*	
		CC	CT	TT	C	T
rs4415084	Cases	16 (24.6)	43 (66.2)	13 (20.0)	75 (52.1)	69 (47.9)
(C>T)	Controls	43 (66.2)	16 (24.6)	6 (9.2)	102 (70.8)	28 (19.4)
		χ^2= 27.004, p= 0.0001*			χ^2= 20.787, p= 0.0001*	

**p* values less than 0.05 were considered statistically significant.

Assessment of the frequency of rs10941679 and rs4415084 among the case and control groups using the chi-square test revealed a significant difference for both rs10941679 and rs4415084. Based on the results, a considerable association between rs10941679 polymorphism and breast cancer was noticed (p= 0.01). In other words, the presence of the G allele resulted in a higher susceptibility to breast cancer (χ^2= 5.411, p= 0.014).

Evaluating the association of different genotypes of rs10941679 polymorphism with breast cancer revealed a significant association of breast cancer with AG genotype (p=0.0003, OR: 1/0.309, 95%CI: 1/0.140 - 1/0.681).

In addition, higher susceptibility to breast cancer was associated with the presence of the T allele for the rs4415084 (χ2= 20.787, p= 0.0001). Also, a considerable relationship between TC and TT genotypes with increased risk of breast cancer was noticed (p-values were 0.006 and 0.001, respectively). Tables 3 and 4 display the genotype and allele frequency of rs10941679 and rs4415084 polymorphisms among the studied population and their association with susceptibility to breast cancer.

Table 4. Association of rs10941679 and rs4415084 polymorphisms with breast cancer risk.

SNPs	Genotype	OR	95% CI	*P* value
rs10941679 (A>G)	AA	1.00	Reference	-
	AG	0.309	0.140 – 0.681	0.0003*
	GG	0.411	0.158 - 1.068	0.065
and rs4415084 (C>T)	CC	1.00	Reference	-
	CT	0.138	0.061-0.312	0.0006*
	TT	0.172	0.056-0.529	0.001*

*Statistically significant ($p < 0.05$)

Discussion

Breast cancer is considered the most prevalent cancer in Iranian women (Mirzaeyan *et al*., 2020). Very little is known about the genetic variations associated with breast cancer susceptibility in the Iranian population. Also, previous studies showed that several genetic variants at the non-coding region of 5q12, are involved with the increased susceptibility to breast cancer. Considering these, in this work, we evaluated the frequency of two major SNPs at 5q12, including rs10941679 and rs4415084 on 72 breast cancer patients and 65 healthy Iranian women. Demographic characterization of patients showed that about 40% of patients had familial cancer history, which shows the increased risk of breast cancer in this group. Previous studies reported the increased susceptibility to breast cancer among women with cancer history in their relatives, which is in agreement with our finding (Mcinerney *et al*., 2009). Also, the majority of patients suffered from ER (75 %) and PR (75 %) breast tumors, while only 26 % of patients had HER-2 positive breast tumors. These results are slightly higher than those reported in a study on Chinese women, that they reported the prevalence of 62 %, 54 %, and 20 % for ER, PR, and ER2 positive breast tumors, respectively (Liang *et al.*, 2016). Thus, we found that ER and PR-positive tumors are frequent breast cancer types in the studied Iranian females who could be considered for therapeutic approaches. Also, the majority of tumors were at pathological grade 1 (52.8 %), without distance metastasis (87.5 %), and with slight involvement of lymph nodes (41.7 %).

The association of rs10941679 and rs4415084 polymorphisms on 5q12 with breast cancer risk in the Iranian population has never been

investigated. In this work, we revealed for the first time that the two low-penetrance loci rs10941679 and rs4415084 on 5q12 confer susceptibility to breast cancer in Iranian females. Also, investigating the association of rs10941679 and rs4415084 with tumor hormone receptors showed no significant association (p= 0.402 and 0.427, respectively). Several studies on the association of the mentioned polymorphisms with breast cancer susceptibility were conducted. However, their results were contradictory. The results from the current work were similar to the results reported by Stacy et al, 2008 in a study on European women. Also, Thomas *et al*. (2009) reported the effect of rs4415084 on breast cancer risk in the EA population. However, contradictory to our results, they found no association between breast cancer susceptibility and rs10941679. Also, contradictory to our results, previous studies on some African and Chinese populations found a borderline or null association between the mentioned polymorphisms and breast cancer risk (Liu *et al*., 2012; Zheng *et al*., 2010; Ruiz-Narvaez *et al*., 2010). The difference could be related to different population sizes and ethnicity. The biological mechanism of breast cancer susceptibility through genetic variation at 5q12 is not well understood. However, Liu et al. in a genome mapping study showed that the *MRPS30* gene, which is located at proximal to rs10941679 and rs4415084 on 5q12, is an important gene involved with cell apoptosis and development of breast cancer. Thus, they hypothesized that the up-regulation of the *MRPS30* gene in response to the SNPs may be involved with breast cancer development. They also hypothesized that the rs4415084 might be associated with some potentially functional polymorphisms, such as rs3761648, which may affect the expression of the *MRPS30* gene and results in breast cancer development. The *MRPS30* gene, which encodes a component of the small subunit of the mitochondrial ribosome, is not expressed in normal breast luminal epithelial cells while overexpressing the infiltrating ductal carcinomas (Grigoriadis, 2006; Liu *et al*., 2012). Also, it was found that the risk allele of rs10941679 was associated with the up-regulation of *FGF10* and *MRPS30* (Ghoussaini *et al*., 2016). Ghoussaini et al. reported that rs10941679 maps to an enhancer element that could interact with the promoter regions of the oncogene *FGF10* and *MRPS30* in breast cancer cells. In addition, Zhang *et al*. (2018) predicted the cis-regulatory involvement of the rs4415084 with breast cancer development by promoting the transcription of *MRPS30* and lncRNA RP11-53O19.1. Therefore, it seems that the rs4415084 and rs10941679 susceptibility loci at the non-coding region of 5q12 are associated with increased breast cancer risk through activation of *FGE10* and *MRPS30*, the key factors in the development of breast cancer.

Conclusion

This work investigated the association of two genetic variants at the non-coding region of 512q, rs4415084, and rs10941679, with breast cancer susceptibility in an Iranian female population. This work revealed that the presence of AG genotype of rs10941679, and TC and TT variants of rs4415084 remarkably increased breast cancer risk. However, further studies with a larger population, focusing on different SNPs could be helpful to elucidate the role of genetic variations at the non-coding regions of 512q in the development of breast cancer in the Iranian population.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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