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Correlation between *Lsp1* (*Rs3817198*) and *Casc* (*Rs4784227*) Polymorphisms and the Susceptibility to Breast Cancer

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Abstract

Background: Breast cancer is classified as one of the common cancers among women worldwide. Within numerous genetic factors involved in the development of breast cancer, *lsp1* and *casc* genes are both located on breast cancer susceptibility locus. While the SNP *rs3817198* in *lsp1* gene has a twilight association with breast cancer in different populations, *casc rs4784227* polymorphisms have been reported to associate with breast tumor appearance in Asian, European, and African ancestry populations. The present report was designed a case-control group aimed at assessing the association of these two SNPs with breast cancer risk in the Iranian population.

Methods: In the case-control study of *rs3817198* and *rs4784227* polymorphisms in 100 women with breast cancer and 100 healthy women were examined by Tetra Arms PCR. Data collected using SPSS software and chi-square test and correlation coefficient were used for statistical analysis.

Results: The results of current study showed that the Chi-square of *lsp1 rs3817198* and *casc rs4784227* polymorphism genotypes in breast cancer, were reported to be 51.613 and 47.920, respectively. Also there has been a significance level of both polymorphisms resulting in the frequency of genotypes in these two polymorphisms between case and control group.

Conclusions: Our finding thus suggested that in both polymorphisms, homozygote genotype showed strong correlation with cancer susceptibility. While, TT genotype in *lsp1 rs3817198* showed significant association with pathogenic properties, in the case of *casc rs4784227* genotypes CC, and in second place, TT showed similar correlation.

Keywords: Breast cancer, *Casc*, *Lsp1*, Polymorphism.

Introduction

According to the GLOBOCAN report, breast cancer is considered one of the top lists of the most abundant female cancers raising from 1.7 to more than 2 million new cases from 2012 to 2018, worldwide. Australia and New Zealand (94.2/100,000), West (92.6/100,000) and North (90.1/100,000) Europe, and North America (84.8/100,000) had the highest age-standardized rates (ASR), whereas the lowest rates were in South-Central Asia (27.9/100,000) and Middle Africa (25.9/100,000) (1). Iranian population ASR has raised from 28.1 to 33.21/100,000 between 2012 to 2018 (2). Environmental variables and germline mutations are two well-known risk factors of breast cancer (4–6). Some known gene locus that is associated with the risk of breast cancer is including *BRCA1*, *BRCA2*, *FGFR2*, *TNRC9/TOX3*, *MAP3K1*, *LSP1*, TNRC9, *PTEN*, *TP53*, *CHEK2*, *ATM*, *BRIP1* and *PALB2* (3-6). While familial genetic-based

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susceptibility of breast cancer accounts for less than 25%, the remaining 75% genetic variance is expected to be due to another form of unknown diversities (5). In some cases, single nucleotide polymorphisms or SNPs can alter mRNA and subsequently protein expression or function, and thereby influence the cells to be susceptible to be malignant.

Leukocyte-specific protein 1 (*lsp1*, located on 11p15.5) gene encoding an F-actin binding protein that expressed in all hematopoietic cells which regulates neutrophil morphology and motility, adhesion to fibrinogen matrix proteins, and transendothelial migration (7). The whole studies investigating the *lsp1* gene polymorphism association and risk of breast cancer showed controversial results. Nevertheless, some studies showed no association between *rs3817198* polymorphism and breast cancer (7-9), some showed a decreasing or increasing trend of breast cancer risk (5, 6, 8-10). TOX3 and FOXA1 proteins are believed to be the other probable candidate which causes breast cancer susceptibility. The *rs4784227* (*casc16*, located on *16q12.1*) polymorphism, which is located at the non-coding part of the gene, possibly may affect the DNA binding sequence change on FOXA1 and subsequently may triggers the FOXA1-binding affinity to the *TOX3* gene promoter (11). Several reports have been shown that *16q12.1* locus, which harbors *rs4784227-CASC16* SNP, has been associated with breast malignancy in Asian, European, and African ancestry populations (5, 12-14). In the present study, we tried to clear the relationship between breast cancer risk and *lsp1* (*rs3817198*) and *casc* (*rs4784227*) gene polymorphism.

Materials and methods

Study population

The total number of 200 samples of 100 healthy and 100 diagnosed breast cancer women were recruited from the Oncology Institute of Imam Khomeini Hospital of Tehran. Breast cancer was diagnosed via clinical and mammography examinations that were further characterized by the histopathological assessment of biopsies. The participants had signed the consent form which was approved by the Ethical Committee of Oncology Hospital.

DNA extraction

The whole blood samples were collected and stored in EDTA (pH= 8) containing tubes at 4 °C for DNA extraction. Then, Genomic DNA was extracted by a DNA extraction kit (Gene Transfer Pioneers Co., Tehran, Iran) according to manufacturer protocol. After the appropriate time, all samples were lastly stored at -20 °C until performing the PCR.

Tetra-Arms PCR

The *lsp1* and *casc* gene DNA region containing identified *rs3817198* T> C and *rs4784227* C> T polymorphism were used to design four sets of PCR primers (Table 1). The primers were designed using OLIGO Primer Analysis, Gene Runner and primer3 software. The PCR reactions were done in a total volume of 10 ml, containing 5 pmol of both inner and outer primers, PCR master mix (Amplicon, Brighton, UK), and 50 ng of DNA. The PCR was performed with 1 cycle of 95 °C for 5 min; 35 cycles of 60 s at 95 °C, 30 s at 62 °C for *lsp*1 and 52 °C for *casc* followed by 30 s at 72 °C; and a

Table 1. PCR primers and conditions for identification of *lsp1* T> C and *casc* C> T polymorphism.

Primer name	Sequence	Genotype pattern (bp)
***LSP1*-FO[1]**	5′-CTCTCACCTTCAACTCTTGGTCTCCTTT-3′	562 bp (outer) 362 bp (C) 249 bp (T)
***LSP1*-RO[2]**	5′-AGTAGGACCTAAGTTCCTGCCCCTCTAT-3′	
***LSP1*-FI[3]**	5′-ACCTGATACCAGATTCAAACTCGCC-3′	
***LSP1*-RI[4]**	5′-CGGGCTGACTTCTAGTGAAATGATCA-3′	
***CASC*16-FO**	5′-ATGAAAGAATACATGAATGAAAAGGAG-3′	423 bp (outer) 273 bp (C) 203 bp (T)
***CASC*16-RO**	5′-AGTCAGTTCCTGGATCAACAAACATTA-3′	
***CASC*16-FI**	5′-AAAAGTCCCAATTTGTAGTGTTTTCC -3′	
***CASC*16-RI**	5′-GATGGGAGTATTTACATCACAATAGCA-3′	

*Forward outer primer, 2. Reverse inner primer, 3. Forward outer primer, 4. Reverse inner primer.

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final extension of 5 min at 72 °C. The PCR products were electrophoresed on a 1% agarose gel, and virtualized with safe stain (SinaGen Co., Iran) using gel Documentation Supply (Fig. 1).

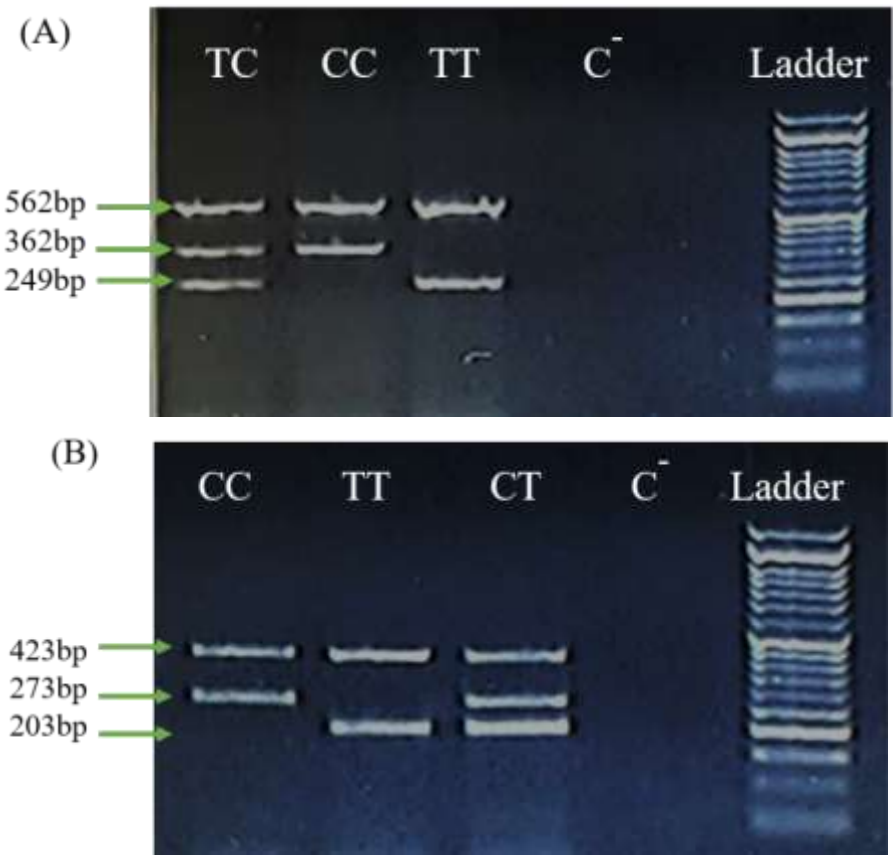

Fig. 1. The SNP *rs3817198* T> C and SNP *rs4784227* C> T genotyping by T-ARMS-PCR resolved on a 1% agarose gel. (A) 562 band represents the common amplicon, whereas the in SNP *rs3817198* T> C T and C allele-specific bands are represented by the 362 and 249 bp amplicons, respectively. (B) Genotyping pattern for common amplicon of SNP *rs4784227* C> T. 423 band represents the common amplicon, whereas the in C and T allele-specific bands are represented by the 273 and 203 bp amplicons, respectively. Ladder represented 50bp DNA Marker.

Statistical Analysis

To investigate allele frequencies and goodness of fit tests for Hardy–Weinberg equilibrium were calculated using the SPSS, Version 22 (; SPSS Inc, IBM Inc., Chicago, IL, USA). The odds ratio (ORs) was calculated by chi-square test to evaluate the association of *lsp1 rs3817198* T and *casc rs4784227* polymorphisms with breast cancer susceptibility. For all analyses, p-value< 0.05 considered as statistically significant.

Results

One hundred patients with breast malignancy and also 100 healthy controls were enrolled in the present study. The frequencies of genotypes andalleles at *rs3817198* T> C and *rs4784227* C>T loci in both patients and controls are illustrated in Table 2. Genotype frequencies in both patients and controls were in accordance with the Hardy-Weinberg equilibrium (p> 0.05). In *rs3817198* locus, among 144 heterozygous individuals, 55.56% (80/144) and 44.44% (64/144) were seen in healthy and patients, respectively. Among 51 normal homozygous genotypes, 60.76% (31/51) were observed in the patients and 39.22% (20/51) was observed in healthy controls. Surprisingly, all patient individuals showed homozygous mutant genotypes (5/5). The frequency of the T allele at this locus was 51.22% (126/246) and 48.78% (120/246) in patients and controls, respectively.

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Also, the frequency of the C allele at this locus was 48.05% (74/154) and 51.95% (80/154) in patients and controls, respectively. There have been significant differences in the frequencies of both genotypes and alleles between breast cancer patients and the control group at this position (p= 0.000, and p= 0.010), respectively. Therefore, there has a significant relationship between homozygous mutant genotype and cancer susceptibility.

Table 2. Frequencies of genotypes and alleles at positions *rs3817198* T/C and *rs4784227* C/T in patients with breast cancer and healthy controls.

Alleles and genotype		Groups			p-value
	Genotype	**Patients**	**Controls**	**Total**	
***lsp1* T> C (*rs3817198*)**	TT	31	20	51	0.000
	CT	64	80	144	0.000
	CC	5	0	5	0.000
		100	100	200	
	Alleles				
	T	126	120	246	0.01
	C	74	80	154	
	Total	200	200	400	
***casc* C> T (*rs4784227*)**	**Genotype**				
	CC	51	10	61	0.000
	CT	36	83	119	0.000
	TT	13	7	20	0.000
		100	100	200	
	Alleles				
	C	138	103	241	
	T	62	97	159	0.000
	Total	200	200	400	

Discussion

Breast cancer is a metastatic malignancy with a strong ability to transfer to distant critical organs like brain, lung, bone, and liver, therefore, ranked as the second cause of death worldwide. The high incidence and mortality of this cancer can be considered as one of the most challenging and controversial health problems worldwide. During the past decade, reports showed a significant rising trend in Asian populations (15). Several studies introduce different risk factors related to breast cancer incidence including environment, lifestyle, hormonal balance, age and familial genetic susceptibility background, however, the pathogenesis of breast cancer is unclear yet (16-20). In recent years, many studies found important roles of genetic factors in the occurrence of breast cancer. The results showed that genetic 5-10% of breast cancer cases in women and 4-40% in men result from inherited mutations in breast cancer susceptibility genes including BRCA1, BRCA2, TP53 and PTEN and in second place, ATM, CHEK2, and PALB2 (21-23). A large proportion of familial cases of breast cancer and possibly single cases are caused by the effects of low-risk alleles, some of which are very high in frequency and likely through multifactorial mechanisms and interact with environmental factors and lifestyle. While there are vigorous methods to evaluate millions of genetic markers in population cohort studies have examined potential risk factors, there have been only a few discovered modifications of low-risk alleles including *FGFR2*, *CASP8*, *LSP1*, *MAPKI* and *TNRC9* in a relatively small number of populations (21, 24).

Genetic related studies of breast cancer have shown the association between single nucleotide polymorphisms in five genes including *FGFR2*, *LSP1*, *H19*, *TOX3 MAP3K1* with susceptibility to breast cancer in European, Asian and American-African populations (6). *LSP1* is mainly expressed in lymphocyte-derivative cells as well as endothelium that is mainly collaborating on the activation and chemotaxis of neutrophils. Several genome-wide studies have been assessed that *lsp1 rs3817198* polymorphism could affect the susceptibility to breast cancer (25). In the

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present work, results showed that there were significant differences in the distribution of CC and in second place TT genotypes in *rs3817198* polymorphism between cases and controls, which were significantly associated with breast cancer susceptibility. This trend is also reported in several papers which mentioned that homozygote genotype is correlated with breast cancer incidence in Caucasians, Asian and African populations (10, 26, 27).

In the European population, genome-wide studies revealed that there is a strong association between chromosome 16 long arms (*16q12.1*) with the occurrence of breast cancer. The *rs4784227* C> T polymorphism is negatively correlated with estrogen receptor non-hereditary breast cancer and hereditary breast cancer (28). Several reports found the significant relation between the upstream locus of TOX3 and FOXA1 with *casc*16 *rs4784227* gene polymorphism and breast tumor susceptibility (12, 14, 29, 30). According to studies conducted by different groups of scientists, the most frequent C allele and the most CC genotype are reported related to *casc*16 *rs4784227* polymorphism. In the present study, the most abundant frequency is shown in heterozygous genotype CT, which is in agreement with part of the studies. In some populations, CT genotype is seen in both healthy and patients. In other studies, however, most of the genotypes were CC homozygotes. In our study, the lowest genotype was related to the homozygous mutant TT, which is consistent with the findings of some in different populations. In contrast with our results, there was a significant association between TT and CT genotypes with the risk of breast cancer in the northwest part of Iran (14).

In overall point of view, the results showed that there were significant differences in the distribution of TT (and in the second place CC) genotype and C allele in *rs3817198* polymorphism as well as CC genotype and C allele in *rs4784227* between cases and controls, which were significantly associated with breast cancer risk.

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