Interleukin-7 receptor gene *rs12516866* polymorphism decreases the susceptibility of estrogen receptor negative Saudi women to breast cancer

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Genet. Mol. Res. 17 (4): gmr16039935

Received Oct 22, 2018

Accepted Oct 25, 2018

Published Nov 05, 2018

DOI: http://dx.doi.org/10.4238/gmr16039935

ABSTRACT.

Background: Genetic polymorphisms in IL-7R are associated with the risk of development of multiple diseases, including breast malignancy. At present, no evidence exists for an influence of gene polymorphism of IL-7R on BC susceptibility in Saudi Arabian populations. The aim of the study was therefore to evaluate whether common polymorphisms in the IL-7R gene (rs1053496 and rs12516866) are correlated with the risk of BC development among Saudi Arabian women.

Methods: In total, 243 samples were collected and divided in to two cohorts: 127 female Saudi patients with BC and 116 age-matched healthy individuals as a control group. Genomic DNA was extracted from peripheral blood samples of individuals of both groups. The IL-7R rs1053496 and rs12516866/T gene variations were genotyped using the TaqMan assay. Odds ratios and 95% confidence interval

were computed from logistic regression models after adjusting for age and estrogen status of the BC group.

Results: The IL-7R gene promoter variant *rs12516866* was correlated with a decreased risk of breast cancer development among the estrogen receptor negative (ER-) participants. The TT homozygote mutant genotype and the T mutant allele provided significant protection against the susceptibility to breast cancer, suggesting that the presence of that genotype and allele is a vital protective factor against BC susceptibility. However, the presence of IL-7R rs1053496 was not significantly associated with BC development in the study population.

Conclusion: Our findings suggest that the IL-7R gene *rs12516866* polymorphism could serve as a potential biomarker for early detection of BC among female Saudi individuals.

Key words: Breast cancer; Interleukin-7; Estrogen; IL-7R; Polymorphism

INTRODUCTION

Female breast cancer (BC) is a major leading cause of malignant disease-related mortality worldwide (Heers H, Stanislaw J, Harrelson J, Lee MW, 2018). Several factors are involved in the initiation and progression of BC; however, the main factors are the heterogeneous character of the disease and its complex genetic features (Wang D et al., 2018). In 2012, BC accounted for 26% of all newly diagnosed malignancy cases in Saudi Arabian women (Bazarbashi S, Al Eid H, Minguet J, 2017). The Saudi Cancer Registry has recently reported a dramatic increase in the mortality and incidence rates of BC and a more aggressive disease, particularly in younger women, when compared to western populations (Bazarbashi S, Al Eid H, Minguet J, 2017; AlJohani B et al., 2016). In addition, most patients with BC patients in Saudi Arabia are admitted to the hospital at a late stage and are diagnosed with advanced phase disease, resulting in greater mortality and morbidity (Al Tamimi DM et al., 2010). Therefore, early detection of BC in Saudi patients is the best strategy for reducing BC mortality rates (Figueiredo F et al., 2018).

The development and progression of BC are now believed to involve pathways associated with cytokines, such as interleukin-7 (IL-7). IL7 is a well-documented inflammatory cytokine that plays a fundamental role in the activation of the human adaptive immune system (Gao J, Zhao L, Wan YY, Zhu B, 2015) and is critical for the proliferation and homeostasis of naïve and memory T cells (Surh CD, Sprent J, 2008). IL-7 also has an apparent association with the progression of human tumorigenesis (Qu H et al., 2016). At the molecular level, the human IL-7 gene is a 72 kb segment located on chromosome 8q12-13 and encodes a 20 kDa protein 177 amino acids in length (Jiang Q et al., 2005). The biological function of this cytokine is mediated through the cell surface interleukin-7 receptor (IL-7R) (Gao J, Zhao L, Wan YY, Zhu B, 2015), a heterodimer complex that contains two distinct chains: IL-7Rα, and IL-7Rγ (Gao J, Zhao L, Wan YY, Zhu B, 2015; Jiang Q et al., 2005). The first chain (IL-7Rα) is highly expressed in humans lymphocytes, such as T/B lymphoid precursors, developing T and B cells, naïve and memory T-cells, and it shares homology with thymic stromal lymphoprotein (TSLP) (Jiang Q et al., 2005). The second chain (IL-7Rγ) is expressed on most hematopoietic cells [10]. The IL-7Rα receptor is attached directly to the Janus kinase 1 (JAK-1) protein, and the Janus kinase 3 (JAK-3) is attached to the cytosolic tail of the γ chain (Swainson L et al., 2007). Binding of IL-7 to its corresponding receptor therefore activates JAK-1 and JAK-3 in the cytosol and the transcription signal transducers and activators of transcription (STAT-5) proteins, leading to phosphorylation of the signal transducer. Phosphorylated STAT (pSTAT) translocates

to the nucleus, where it activates anti-apoptotic genes, such as Bcl-2 and Mcl-1, and inhibits pro-apoptotic proteins, such as Bax and Bak (Swainson L et al., 2007) thereby preventing apoptosis and promoting BC development and progression.

IL-7R mRNA is highly expressed in the breast, colon, lung, colon and renal cell carcinomas (Cosenza L, Gorgun G, Urbano A, Foss F, 2002) . Al-Rawi et al. showed significantly increased levels of IL-7 and IL-7R expression in patients with BC (Al-Rawi MA et al., 2004) and in human lung cancer cells (Ming J, Zhang Q, Qiu X, Wang E, 2009) Suzuki et al.reported a linkage between higher expression of IL-7R and worse outcome in patients with stage I lung adenocarcinoma (Suzuki K et al., 2013). At present, the role of IL-7 in BC is unclear, as it could either favor the progression of BC or have an inhibitory effect (Capitini CM, Chisti AA, Mackall CL, 2009)

The development of many malignancies is associated with single nucleotide polymorphisms (SNPs), which are responsible for individual variations (Gerger A et al., 2010). Genetic polymorphisms in the IL 7R gene are associated with an increased risk of diverse diseases, including inflammatory responses, autoimmune diseases, and malignancies (Mazzucchelli RI, Riva A, Durum SK, 2012) such as T-cell leukemia, (Zenatti PP, lymphomas et al., 2011; Ming J, Zhang Q, Qiu X, Wang E, 2009) and BC (Gerger A et al., 2010). A previous study in Caucasians identified two SNPs (rs1494555 and rs7737000) in IL-7R that were correlated with an increased risk of lung cancer, and polymorphism of rs7737000 was associated with the risk of lung cancer in African Americans (Van Dyke AL et al., 2009). In addition, a variant of rs6897932 in the IL-7R gene was linked with susceptibility to autoimmune diseases (Wang XS et al., 2014). Detection of SNPs that can cause cancer can assist clinicians in identifying at-risk patients, which can then provide better individual treatment and early diagnosis of patients with BC. Until now, no evidence has been presented to support the influence of gene polymorphism of IL-7R on the BC susceptibility of Saudi Arabian populations. The aim of the current study was therefore to evaluate the possible correlation between common IL-7R polymorphisms (rs1053496 and rs12516866) and BC development in Saudi Arabian women.

MATERIALS AND METHODS

Ethical statement

Prior to the initiation of the present study, ethical approval was obtained from the Ethical Review Committee located at King Faisal Medical City (KFMC) in Riyadh, Saudi Arabia (ethical approval number 15-089E). A consent form was designed before the beginning of the study and the participants who signed the written consent form were asked to answer a self-administered questionnaire, which included their approval to publish the data generated from this study. The evaluated clinicopathological parameters included the participant's age, tumor site, tumor characteristics, histological subtype, family history of malignancy, individual history of malignancy, and number of pregnancies and deliveries. Immunohistochemical methods were also used by Dr. Maha Arafah, a pathologist specialist in King Khaled hospital, to evaluate the status of the estrogen receptor (ER) and progesterone receptor (PR) in each participant.

Characteristics of the study subjects

This population case-control study was conducted using 127 blood specimens collected from women diagnosed with BC (the case group) and 116 blood specimens from healthy women who had no history of any malignancy and had a normal mammography test at the time of sample collection (the control group). All blood specimens were collected by Dr. Al Naeem Abdurahmane and Dr. Sanae Abdullah Ajaj between 2011 and 2016 at the King Faisal Medical City and King Saud University Hospital in Riyadh, Saudi Arabia. Control subjects were frequency aged-matched to the BC patient subjects (±5 years). The clinical and demographic characteristics of the patients with BC and the healthy control subjects are shown in Table 1.

Table 1. Demographic and clinic-pathologic characteristics of the healthy controls and patients with breast cancer.

Variables	Demographics	Case Patients	Control Patients
Age (Years)	≤48	45	62
Median age (48±8.2*)	>48	82	54
Estrogen receptor status	ER+	76	-
	ER-	49	-
Progesterone receptor status	PR+	71	-
	PR-	55	-
HER Status	HER+	49	-
	HER-	78	-
Total individuals	-	127	116

Inclusion and exclusion criteria

Inclusion criteria in the present study include patients' age between 28 and 60 years old, female gender, Saudi ethnic, no individual history of malignancy, no significant differences in total number of individuals among breast cancer cases and healthy controls, and all samples are collected from individuals live in Riyadh region. However, the subject's previous treatment history, a previous individual cancer history or/and previous diagnosis of inflammatory autoimmune disease, patients consuming cigarette smoking or drinking alcohol, participants who do not sign the written consent form, and existing of family history of malignancy are excluded from the current study.

Blood samples and DNA extraction

After evaluating the clinicopathological data, 3 ml of whole blood was collected from all participants into EDTA tubes. Genomic DNA was isolated from 200 µl of all the whole blood samples using a QIAamp kit according to the manufacturer's recommended protocol. The DNA samples were then preserved at -20oC until use. The concentration of isolated DNA in each sample and its purity were quantified at A260/A280 nm and A260/A230 nm using a NanoDropTM 8000 spectrophotometer.

Genotyping for the IL-7R gene polymorphisms

The rs1053496 and *rs12516866* polymorphisms in the IL-7R 3′ -untranslated region (3'-UTR) and promoter, respectively were genotyped using the 96-well plate Taq Man assay and an ABI 7500 Real Time PCR machine (Applied Biosystems, Foster City, CA, USA).These polymorphisms were selected based on their positions and frequencies in cancer reported in literature studies. The fragments were amplified in 96-well plates with forward and reverse primers using the following cycling parameters: initial incubation period of 7 min at 95°C; 40 cycles of 40 seconds at 95°C, 40 seconds at 60°C, and 40 seconds at 72°C; and a final extension period of 7 min at 72°C to complete the PCR reaction. Each well contained 10 to 20 ng of DNA, 5.6 of 2× TaqMan Genotyping Master Mix and 200 nM of in a total volume of 10 µl. The PCR primers and reagents were provided by Applied Biosystems (USA). Efficiency was evaluated in both patient and control samples using positive and negative controls.

Statistical analysis

Differences in the allele and genotype frequencies for each SNP were analyzed using the Chi–square Hardy-Weinberg equilibrium (HWE) test. The correlation between IL-7R polymorphisms and BC risk were evaluated using logistic regression to calculate both the odds ratios (ORs) and the 95% confidence intervals (CIs) and compared with the control subjects.Two sided P values less than 0.05 were considered statistically significant. All data analyses were conducted using the SPSS version 16.0 statistical package software (SPSS, Chicago, USA).

RESULTS

Demographic characteristics of study subjects

In total, 243 specimens from two cohorts (patients with BC and healthy controls) were analyzed. Both the patients and healthy controls were Saudi females who voluntarily participated in the study. The first cohort comprised 127 patients with BC with a median age of 48±8.2 years at diagnosis who had been admitted to the Oncology Centre at King Faisal Medical City between 2014 and 2017. Of these patients, 45 were younger than 48 years old and 82 were older than 48 years old. We evaluated the clinicopathological features of the patients with BC according to their ER, PR, and human epidermal growth factor receptor (HER) statuses. Of these patients, 76 were ER+, 49 were ER-, 71 were PR+, 55 were PR-, 49 were HER+, and 78 were HER-.

The second cohort consisted of a total of 116 healthy women controls. No significant difference was noted in the age distribution between the patients with BC and the healthy controls. However, the participant in the second cohort was considered healthy if they and all family members had no history of BC. In the control group, 62 participants were younger than 48 years old and 54 were older than 48 years old. Various clinical characteristics of the patients with BC and the healthy controls were recorded, such as cancer duration, family BC history, BC grade, cigarette smoking habits, and presence of other diseases. Table 1 summarizes the clinical characteristics of the BC patients and the normal controls. Global analysis of allelic and genotypic frequency prevalences in IL-7R rs1053496 and *rs12516866* polymorphisms and their association with BC development.

As shown in Table 2, the two IL-7R gene SNPs were selected based on specific criteria. The rs1053496 C/T is a 3'-UTR variation located at NC_000005.10:g.35879327, while the *rs12516866* G/T is a promoter region variation at NC_000005.10:g.35851159. We investigated the potential association between these two IL-7R gene SNPs and the risk of BC development in the adult female Saudi Arabian population. As shown in our global statistical analysis, we found no significant associations between either SNP frequencies in the BC and control groups.

The genotypic distributions of *rs12516866* in the BC cohort were 44%, 42%, and 14% for the GG, GT, and TT genotypes, respectively. In the controls, these distributions were 41%, 39%, and 20% for the GG, GT, and TT genotypes, respectively. By contrast, for rs1053496, the genotypic allocations in the BC cohorts were 7%, 42%, and 51% for the CC, CT, and TT genotypes,compared with 6%, 42%, and 52% for the healthy controls, respectively. Table 3 summarizes the allele and genotype frequencies of the IL-7R gene SNPs in adult female Saudi patients with BC and the healthy participants.

Table 2. Description of thes elected IL-7Rgene single nucleotide polymorphisms (SNPs).

SNP ID	SNP location	Variation type	Alleles change
rs1053496	NC_000005.10:g.35879327	3'-UTR	C/T
rs12516866	NC_000005.10:g.35851159	Promoter	G/T

Table 3. Genotype and allele frequencies of IL-7R gene single nucleotide polymorphisms (SNPs) in female Saudi patients with breast cancer and healthy controls.

SNP	Genotype /allele	Breast	Control	OR	95% CI	X^2	P value
rs12516866	**total**	**159**	**184**				
	GG	70(0.44)	76(0.41)	Ref			
	GT	67(0.42)	72(0.39)	1.0103	0.6346-1.60825	0.001872	0.96548
	TT	22(0.14)	36(0.20)	0.6634	0.3562-1.23572	1.681261	0.18718
	GT+TT	89(0.56)	108(0.59)	0.8947	0.5825-1.37421	0.258277	0.61137
	G	207(0.65)	224(0.61)	Ref			
	T	111(0.35)	144(0.39)	0.8341	0.6109-1.13892	1.303732	0.2521
rs1053496	**total**	**159**	**188**				
	CC	11(0.07)	12(0.06)	Ref			
	CT	66(0.42)	79(0.42)	0.911	0.377-2.199	0.04262	0.836773
	TT	82(0.51)	97(0.52)	0.922	0.386-2.199	0.03334	0.855382
	CT+TT	148(0.93)	176(0.94)	0.917	0.393-2.139	0.03987	0.842087
	C	88 (0.28)	103(0.27)	Ref			
	T	230(0.72)	273(0.73)	0.986	0.705-1.377	0.00673	0.9346

Distribution of the genotype and allele allocation correlation of IL-7R gene SNPs in patients with BC and healthy controls based on clinical parameters.

We examined the effect of IL-7R polymorphisms on diverse clinical features of patients with BC by analyzing the allele and genotype distributions of the IL-7R genetic variations and a series of clinicopathological parameters, including the age and ER status for BC cases and normal subjects. We first investigated the correlations of the IL-7R gene polymorphisms and participant ages at the time of BC diagnosis by segregating the patients with BC and controls into groups either younger than 48 years old or older than 48 years old. Table 4 presents the comparisons of IL-7Rgene polymorphisms between the healthy volunteers and the patients with BC based on patient age older and younger than 48 years. Neither the *rs12516866* nor the rs1053496 polymorphism showed any significant correlation with the risk of BC among Saudi women younger than 48 years when compared with the healthy populations of the same age (all $p>0.05$). However, the genotypic frequencies in the patients with BC for the *rs12516866* polymorphism were 40%, 44%, and 16% for the GG, GT, and TT genotypes, respectively; they were 45%, 36%, and 19% in the controls for the GG, GT, and TT genotypes, respectively. The G and T allele frequencies were similar in this sub-study population.

Table 4. Genotype and allele frequencies of *IL-7R*gene single nucleotide polymorphisms (SNPs) in female Saudi patients with breast cancer and healthy controls according to the patient's age

A) Aged below 48 years old

SNP	Genotype /allele	Breast	Control	OR	95% CI	X^2	P value
rs12516866	**total**	**75**	**91**				
	GG	30(0.40)	41(0.45)	Ref			
	GT	33(0.44)	33(0.36)	1.36667	0.69639-2.68209	0.826341	0.362058
	TT	12(0.16)	17(0.19)	0.96471	0.40164-2.31717	0.00646	0.93586
	GT+TT	45(0.60)	50(0.55)	1.23	0.66191-2.28565	0.429202	0.511154
	G	93(0.62)	115(0.63)	Ref			

	T	57(0.38)	67(0.37)	1.052	0.67307-1.64425	0.049497	0.8240
rs1053496	**total**	**75**	**93**				
	CC	6(0.08)	3(0.03)	Ref			
	CT	30(0.40)	41(0.44)	0.365	0.084-1.581	1.92346	0.145316
	TT	39(0.52)	49(0.53)	0.397	0.093-1.693	1.639748	0.177531
	CT+TT	69(0.92)	90(.97)	0.383	0.092-1.587	1.866439	0.150711
	C	42(0.28)	47(0.25)	Ref			
	T	108(0.72)	139(0.75)	0.869	0.534-1.413	0.318096	0.5737

B) Aged above 48 years old

SNP	Genotype /allele	Breast	Control	OR	95% CI	X^2	P value
rs12516866	**total**	**84**	**93**				
	GG	40(0.48)	35(0.38)	Ref			
	GT	34(0.40)	39(0.42)	0.7628	0.3998-1.45535	0.675799	0.40996
	TT	10(0.12)	19(0.20)	0.4605	0.1891-1.12147	2.976994	0.07342
	GT+TT	44(0.52)	58(0.62)	0.6637	0.3645-1.20885	1.801934	0.17772
	G	114(0.68)	109(0.59)	Ref			
	T	54(0.32)	77(0.41)	0.6705	0.4336-1.03685	3.243407	0.0693
rs1053496	**total**	**84**	**95**				
	CC	5(0.06)	9(0.10)	Ref			
	CT	36(0.43)	38(0.40)	1.705	0.521-5.574	0.7915	0.35745
	TT	43(0.51)	48(0.50)	1.612	0.501-5.185	0.650936	0.40406
	CT+TT	79(0.94)	86(0.90)	1.653	0.531-5.144	0.766745	0.36327
	C	46(0.27)	56(0.29)	Ref			
	T	122(0.73)	134(0.71)	1.108	0.699-1.757	0.191661	0.6609

The rs1053496 SNP was genotyped in the BC cohort as 8%, 40%, and 52% for the CC, CT, and TT genotypes, respectively, and as 3%, 44%, and 53% in the healthy controls for the GG, GT, and TT genotypes, respectively. The G and T allele distributions were similar between the patients with BC and controls younger than 48 years old (Table 4A). Conversely, the participants older than 48 years showed that no significant correlations in the genetic variations of either polymorphism of IL-7R gene. The frequencies of the GG, GT, and TTgenotypes for the *rs12516866* variant were 48%, 40%, and 12% respectively in the BC cohort and 38%, 42%, and 20% in the healthy controls. However, the distributions of the ancestral genotype CC, heterozygote mutant CT, and double homozygote mutant TT for the rs1053496 SNP were 6%, 43%, and 51%, respectively, in the BC cohort and 10%, 40%, and 50%, respectively, in the control cohort (Table 4B).

We also analyzed the IL-7R gene SNPs according to the ER status of the patients with BC and the healthy controls. The allele and genotype distributions and the analysis for the individual IL-7R SNPs are shown in Table 5A for ER+ patients and in Table 5B for ER- patients. We found no significant differences in the genotypes and allele prevalences between the ER+ subgroup ($p > 0.05$) for *rs12516866* and rs1053496 or for the risk of BC development (Table 5A). The ER+ participants had genotypic prevalences for *rs12516866* of 41%, 40%, and 19% for GG, GT, and TT, respectively, in the BC cohort, compared with 41%, 39%, and 20%, respectively, in the control population. The prevalences of genotypes and alleles for rs1053496 in the BC cohort were 8% for the CC wild homozygote genotype, 33% for the CT heterozygote mutant genotype, and 59% for the TT homozygote mutant genotype, compared with 6% for CC, 42% for CT, and 52% for TT in the healthy subjects (Table 5A).

Table 5. Genotype and allele frequencies of IL-7R gene single nucleotide polymorphisms (SNPs) in female Saudi patients with breast cancer and healthy controls according to estrogen status

A) Estrogen positive

SNP	Genotype /allele	Breast	Control	OR	95% CI	X^2	P value
	total	**87**	**184**				
	GG	36(0.41)	76(0.41)	Ref			
	GT	34(0.40)	72(0.39)	0.99691	0.56442-1.7608	0.000113	0.991502
rs12516866	TT	17(0.19)	36(0.20)	0.99691	0.49507-2.00746	7.49E-05	0.993092
	GT+TT	51(0.59)	108(0.59)	0.99691	0.59397-1.67321	0.000137	0.990666
	G	106(0.61)	224(0.61)	Ref			
	T	68(0.39)	144(0.39)	0.9979	0.6896-1.44404	0.000124	0.9911
	total	**87**	**188**				
	CC	7(0.08)	12(0.06)	Ref			
	CT	29(0.33)	79(0.42)	0.62929	0.22585-1.7534	0.793945	0.398721
rs1053496	TT	51(0.59)	97(0.52)	0.90133	0.33428-2.43029	0.042169	0.839108
	CT+TT	80(0.92)	176(0.94)	0.77922	0.29571-2.0533	0.255741	0.624879
	C	43(0.25)	103(0.27)	Ref			
	T	131(0.75)	273(0.73)	1.14942	0.76102-1.73604	0.438481	0.5023

B) Estrogen negative

SNP	Genotype /allele	Breast	Control	OR	95% CI	X^2	P value
	total	**66**	**184**				
	GG	32(0.48)	76(0.41)	Ref			
	GT	29(0.44)	72(0.39)	0.9566	0.52656-1.73783	0.021223	0.884126
rs12516866	TT	5(0.08)	36(0.20)	0.32986	0.11864-0.91711	4.839422	**0.006869***
	GT+TT	34(0.52)	108(0.59)	0.74769	0.42498-1.31543	1.020702	0.314723
	G	93(0.70)	224(0.61)	Ref			
	T	39(0.30)	144(0.39)	0.65233	0.42497-1.00133	3.846483	**0.0418***
	total	**66**	**188**				
	CC	4(0.06)	12(0.06)	Ref			
	CT	33(0.50)	79(0.42)	1.25316	0.37657-4.17032	0.135772	0.701429
rs1053496	TT	29(0.44)	97(0.52)	0.89691	0.26873-2.99349	0.031331	0.862481
	CT+TT	62(0.94)	176(0.94)	1.05682	0.32864-3.3984	0.008601	0.925229
	C	41(0.31)	103(0.27)	Ref			
	T	91(0.69)	273(0.73)	0.8374	0.54323-1.29086	0.646816	0.4289

In the ER- participants, the TT homozygote mutant genotype and the T mutant allele of SNP *rs12516866* provided significant protection against the BC risk in the Saudi patients, suggesting that the presence of these genotype and alleles is a vital factor in the protection against BC susceptibility (Table 5B). The frequency of the TT genotype was 8% in the patients with BC and 20% in the healthy controls (OR= 0.329; 95% CI: 0.11864-0.91711, and p= 0.006869). However, the T allele was distributed as 30% in the BC cases and 39% in the healthy controls (OR= 0.652; 95% CI: 0.42497-1.00133, and p= 0.0418). By contrast, for rs1053496 ER-individuals, the genotypic allocation showed no significant risk in the BC when compared to the healthy individuals. The genotypic frequency results for rs1053496 in the BC cohort were 6%, 50%, and 44% for CC, CT, and TT, respectively, whereas these were 6%, 42%, and 52%, respectively, in the healthy controls (Table 5B).

DISCUSSION

BC is recognized as a complex multifactorial disease that potentially occurs with genomic instability and DNA damage induced by environmental factors and genetic elements (Pongsavee M,

Wisuwan K., 2018) Mammography screening is currently the best tool for BC diagnosis and is associated with BC mortality reduction (Weedon-Fekjaer H, Romundstad PR, Vatten LJ, 2014). However, the number of BC-related deaths among women is increasing every year in the Kingdom of Saudi Arabia (Semlali A et al., 2017). Genetic differences in some immune-related genes seem to drive inter-individual changes in susceptibility to carcinoma of the breast (Zhifu Y et al., 2015) As common genetic variations, SNPs have associations with several complex diseases (Kosaloglu Z et al., 2016).

The IL 7 gene, through IL-7R, provides signals that are essential for development and maintenance of many stages of human lymphocytes (Kasai H et al., 2018) IL-7R genetic polymorphism is also correlated with susceptibility and prognostic markers in breast carcinogenesis in another SNP not tested in the current study (Vitiello GAF et al., 2018). Our aim in the present study was to assess the possible relationship between IL-7R polymorphisms and risk of BC susceptibility in a large Saudi Arabian cohort of 127 BC cases. To the best of our knowledge, this is the first study to identify a correlation between genetic variations of the IL-7R gene and the BC susceptibility and clinical results among Saudi women. Consequently, these IL-7R SNPs may be useful as potential markers for predicting the overall increase in BC risk in female Saudi patients. Interestingly, we identified that the IL-7R SNP *rs12516866* (located within the promoter region) could be a vital factor in protection against BC susceptibility, and it protects Saudi females through its ER negative feature specifically in double mutant TT variants. Therefore, we conclude that the *rs12516866* promoter variant may be correlated with a reduced BC risk.

In our study, we found no evidence to support an association between IL-7R rs1053496 (located within the 3'-UTR region) and BC risk in our study population. IL-7R is a subunit of the thymic stromal lympho-protein receptor (TSLP R), which is known to contribute to the immunopathology of autoimmune inflammation diseases, such as cancer, that show dysregulated Th2 cell-type cytokine production. Elevated TSLP expression or activation of the heterodimer the complex TSLPR/IL7R signaling pathway is known to promote the growth and metastasis of colon, breast, and pancreatic tumors (Grivennikov SI, Greten FR, Karin M, 2010) The mutations or genetic changes within the IL7 R promoter would influence IL7R gene expression as well as the accessibility of the IL-7R promoter to transcriptional factors, thereby reducing the synthesis of Th2 cytokines induced by the TSLP gene. The balance between synthesis and degradation of the Th2 cytokines will play a key role in the initiation or protection against cancer in general.

The IL7R rs1053496 is located in the 3’ UTR; therefore, this polymorphism can contribute to the stability of IL7R mRNA but not to its expression. To date, no previous studies have evaluated the association between genetic variations of IL-7R *rs12516866* and rs1053496 and cancer, including BC, nor have many studies tested for associations between other SNPs not tested in the present study and diverse diseases such as cancer. One previous study showed that neither the IL-7R rs1494555 nor rs7737000 variants contributed to susceptibility to non-small cell lung cancers in non-smoking Chinese individuals (Bao WL et al., 2011). However, both SNPs contributed to an increased risk of lung cancer in Caucasian women and in African-American women (Van Dyke AL et al., 2009) In addition, other work revealed a connection between the IL7R rs6897932 polymorphism and the progression of liver fibrosis in patients with chronic hepatitis C (Jimenez-Sousa MA et al., 2018) as well as in patients with multiple sclerosis (Sayad A et al., 2017; Majdinasab N, Hosseini Behbahani M, Galehdari H, Mohaghegh M, 2014). Kim et al. (2013) demonstrated a possible contribution of IL-7R polymorphisms to the development of acute leukemia (Kim MS et al., 2013) A previous study suggested that the IL-7R rs1389832, rs1494555, and rs1494556 variants may be related to gastric cancer etiology (Mahajan R et al., 2008).

CONCLUSION

A significant association was found between the IL-7R gene promoter variant *rs12516866* and a decreased risk of BC development among the estrogen receptor negative participants. Therefore, our findings suggest that this polymorphism could be used as a potential biomarker for early detection of BC among female Saudi individuals. Our findings also hold promise for further investigation of the effects of IL7R gene polymorphisms on other cancer types and in other ethnic populations. The strength of our study lies in its uniqueness and novelty. It is the first and distinct study which is interested in the association

between IL7R polymorphism and BC progression in a specific ethnic group who suffers from numerous genetic diseases caused by consanguinity. However, the study has some limitation as well, for instance; collection of vast number of samples and other ethnic groups' studies are required to find any other relationship between IL-7R SNPs and BC to confirm results of the study. Finally, to properly understand the effect of SNPs on breast cancer, it is suggested to study the function of polymorphisms on BC cell proliferation, migration etc…..

List of used abbreviations

BC	Breast Cancer
IL-7	interleukin-7
IL-7R	interleukin-7 receptor
TSLP	Thymic stromal lymphoprotein
TSLPR	Thymic stromal lymphoprotein Receptor
JAK-1	Janus kinase 1
JAK-3	Janus kinase 3
STAT	Signal transducers and activators of transcription signal transducer.
SNPs	Single nucleotide polymorphisms
SPSS	Statistical Package of the Social Sciences
OR	Odds ratio
CIs	Confidence intervals
ER	Estrogen Receptor

CONFLICTS OF INTEREST

All authors approved the manuscript and declare no conflict of interest.

ACKNOWLEDGMENTS

This project was supported by the research group program (number RGP-VPP-260) in the Kingdom of Saudi Arabia.

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