RESEARCH ARTICLE

Lack of Association of *BRCA1* and *BRCA2* Variants with Breast Cancer in an Ethnic Population of Saudi Arabia, an Emerging High-Risk Area

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Abstract

Incidence of breast cancer shows geographical variation, even within areas of ethnic homogeneity. Saudi Arabia has witnessed an increase in occurrence of breast cancer in its unexplored ethnic populations over the past few years. We aimed at determining whether any association exists between single nucleotide polymorphisms in breast cancer associated gene 1 (*BRCA1*) and breast cancer associated gene 2 (*BRCA2*) and the risk of breast cancer. TaqMan based Real Time Polymerase chain reaction genotyping assays were used to determine the frequency of single nucleotide polymorphisms in *BRCA1* (rs799917) and *BRCA2* (rs144848) in a group of 100 breast cancer patients and unaffected age matched controls of Saudi Arabian origin. The present data revealed that neither *BRCA1* nor the *BRCA2* studied variant show any significant association with the disease. This study failed to find any role of the concerned variants in breast cancer either as risk or as prognostic factors. The small number of patients registered was one of the limitations of this study. In summary, comparison of mutation profile with other ethnic populations and regions reflected both differences and similarities indicating co-exposure to a unique set of risk factors. The differences could be due to exposure to particular environmental carcinogens; different lifestyle, reproductive pattern; dietary or cultural practices of Saudi Arabian women that need further investigations.

Keywords: *BRCA1* - *BRCA2* - single nucleotide polymorphism - breast cancer - Saudi Arabia

Asian Pac J Cancer Prev, **14 (10)**, 5671-5674

Introduction

Breast cancer is the most common cause of cancer in women in the Kingdom of Saudi Arabia, the most common cause of cancer death (National Statistics). Breast cancer shows geographical variation in its incidence, even within areas of ethnic homogeneity. Saudi Arabia, over past few years, witnesses an increase in incidence and occurrence of breast cancer in its unexplored ethnic population. The differences could be due to exposure to particular environmental carcinogens; different lifestyle, reproductive pattern; dietary or cultural practices of Saudi Arabian women that need further investigations. In Saudi Arabian women, breast cancer constitutes 18% of all cancers, whilst locally advanced breast cancer disease is unusual in Western countries, which constitutes more than 40% of all non-metastatic breast cancer in Saudi Arabia (Ezzat et al., 1999). In Saudi population 21% of total female cancer diagnosis refers to breast cancer. Age-adjusted breast cancer incidence rates in Western countries are about five times higher than rates in less developed countries (Bin Amer et al., 2008). However, the 2002 annual report of Saudi National Cancer Registry enlighten the fact that breast cancers developed before the age of 40 comprise 26.4% of all female breast cancers comparing to 6.5% in the USA. Since, young age in female had been reported as an independent risk factor for breast cancer in the Saudi population (Elkum et al., 2007). The risk of breast cancer is raised about two fold in the affected Saudi women. Several studies have identified an association with SNPs and breast cancer in the Chinese population (Ma et al., 2006).

It has been proposed that different genetic backgrounds due to the combination of environmental could explain the remaining familial breast cancer risk (Pharoah et al., 2002). Single nucleotide polymorphisms (SNPs) have emerged as genetic markers of choice because of their high density and relatively even distribution in the human genomes (Kruglyak et al., 1999; Venter et al., 2001) and are being used for fine mapping of disease loci and for candidate gene association studies. *BRCA1* and *BRCA2* both accounts for around 25% of families with hereditary breast cancer (Ford et al., 1998). The involvement of *BRCA1* SNPs into onset of breast cancer has been

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studied and had been established that they contribute to individual's susceptibility for breast cancer (Huo et al., 2009). Furthermor, there are reports for the association of *BRCA2* variants with the breast cancer (Song et al., 2006).

rs799917 is a nonsynonymous SNP located in the coding region of *BRCA1*. The C→T variation of rs799917 leads to an amino acid change from proline to leucine at position 871 in the BRCA1 protein. This is a nonconservative change as proline conveys unique structural properties to the polypeptide. This polymorphism lies in the middle of a strongly conserved region of the gene as measured by phastCons analysis of >28 species. rs799917 has been studied for their association with breast cancer in general and sporadic breast cancer specifically, in many population based studies (Cox et al., 2005a; Huo et al., 2009).

rs144848 is also a nonsynonymous SNP. That is located in exon 9 of *BRCA2*. G→T variation of rs144848 leads to an amino acid change from histidine to asparagine at position 372 in the BRCA2 protein (Wu et al., 2004). rs144848 has been studied earlier for their association with non-Hodgkin's lymphoma (Jiao et al., 2012), prostate cancer risk (Agalliu et al., 2010) and familial breast Cancer (Sehl et al., 2009).

The aim of this study was to determine whether previously identified breast cancer susceptibility alleles are associated with breast cancer in the Saudi Arabian women and secondly to ascertain whether there are susceptibility alleles that predispose to breast cancers. Despite the strong hypothesis for an involvement of these genes, to the best of our knowledge the association between these polymorphisms and the development of breast cancer in Saudi population has not yet been investigated. We genotyped *BRCA1* (rs799917) and *BRCA2* (rs144848) SNPs and have shown to predispose in hundred breast cancer cases and age matched controls from the Saudi Arabia.

Materials and Methods

Study group and study design

In this study, hundred breast cancer patients who were clinically and histopathologically diagnosed at King Khalid University Hospital from January 2010-December 2011 were included. All patients underwent complete staging procedures in the respective pathology laboratories. The study was approved by the Ethical-Committee Board of King Saud University. Written informed consent was obtained from all patients and control subjects included in this study.

Sample collection

About 5 mL of venous blood was drawn using a sterile heparinized syringe from healthy individuals as control samples after clinical checkups. The blood samples of malignant cases were obtained after diagnosis from the pathology laboratory for genotyping.

*Extraction of DNA with automated **QIAcube** extraction system*

Genomic DNA was extracted using QIAamp mini blood DNA extraction kit (QIAgen, Netherland) as per the instructions of the manufacturer.

*Determination of BRCA1 (rs799917) and BRCA2 (rs144848) Polymorphic status of Breast Cancer by **TaqMan** Real Time PCR*

The determination of genotypes was performed exactly as earlier publication (Alshatwi et al., 2011). However, here we describe that briefly. Genotyping was carried out using TaqMan (Applied Biosystems, California, USA) technology according to the manufacturer's instructions. Primers and probes were supplied directly by Applied Biosystems as Assays-by-Design™ . All assays were carried out in 96-well plates. Cases and controls were arranged in a chequer board pattern on each plate, to ensure even treatment during the assay procedure and each plate included negative controls (with no DNA). Plates were read on the ABI Prism 7500 Fast using the Sequence Detection Software (Applied Biosystems, California, USA) using the following conditions: 50°C for 2 minutes, 95°C for 10 minutes, and then 40 cycles of amplification (92°C denaturation for 15 seconds, 62°C annealing/extension for 60 seconds). Failed genotypes were repeated. Assays in which the genotypes of duplicate samples did not show >95% concordance were discarded and replaced with alternative assays with the same tagging properties.

Statistical analysis

For each SNP, deviation of genotype frequencies in cases and controls were compared by χ^2 test for heterogeneity (two degrees of freedom) and test for trend (one degree of freedom), In order to evaluate the ethnicity-specific effect. Genotype specific risks were estimated as odds ratio (ORs) using unconditional logistic regression. No statistically significant differences were found (data not shown) and so the results have been combined and the risk associated with each SNP was estimated by allelic, dominant and recessive ORs and associated 95% confidence intervals. ORs with 95% confidence intervals were calculated to assess the strength of the association between polymorphism and breast cancer risk. We explored the association for codominant model, dominant model, recessive model and allele versus allele, respectively using a threshold of $p \leq 0.05$. All statistical tests were based on two-sided probabilities using SPSS V.19 (Armonk, New York).

Results

Characteristics of patients

The clinicopathological characteristics revealed early onset and late diagnosis of breast cancer in this scenario (Table 1). The average age of the randomly selected group of invasive ductal carcinoma cases, with stage I (24%), stage II (39%) and stage III (33%) disease was about 51.5 years. 58% of the patients were pre-menopausal cases with an average age of 37.5 years. The average age of post-menopausal patients was found to be 61.2 years.

***BRCA1** (rs799917) polymorphism*

As shown in Table 2, the genotypic frequencies

Table 1. Characteristics of Breast Cancer Patients (%) showing BRCA1 and BRCA2 Gene Polymorphism Categorized by Age, Tumor Stage and ER Status

Tumor Stages (%)					ER Status (%)		Age Group (%)		
I	II	III	IV	UD	Positive	Negative	Undermined	Pre-menopausal (Average age 37.5years)	Post-menopausal (Average age 61.2years)
24	39	33	2	2	63	31	6	58	42

Table 2. Distribution of BRCA1 and BRCA2 Genotypes and Allelic Frequencies of the Study Population

BRCA1 (rs799917)	Genotypes			Allelic Frequency	
	CC	CT	TT	C	T
Patients	31	37	32	0.49	0.51
Controls	30	36	34	0.48	0.52
	χ^2=0.17, d.f.=2, p=0.95			χ^2=0.18, d.f.=1, p=0.76	
BRCA2 (rs144848)	Genotypes			Allelic Frequency	
	GG	GT	TT	G	T
Patients	38	33	29	0.54	0.45
Controls	33	32	35	0.49	0.51
	χ^2=1.81, d.f.=2, p=0.40			χ^2=2.42, d.f.=1, p=0.11	

Table 3. Odds Ratio with 95% CI of BRCA1 and BRCA2 Gene in Breast Cancer Patients

Genotypes		Odds Ratio (CI 95%)	p value
BRCA1 (rs799917)	CC vs CT	1.23 (0.48-3.18)	0.641
	CC vs TT	0.04 (0.74-5.68)	0.127
	TT vs CT	1.66 (0.74-3.72)	0.177
Dominant Model	CC vs CT+TT	1.51 (0.63-3.65)	0.318
Recessive Model	TT vs CT+TT	0.57 (0.27-1.20)	0.106
	C/T	1.06 (0.71-1.57)	0.764
BRCA2(rs144848)	GG vs GT	1.11 (0.56-2.19)	0.748
	GG vs TT	1.13 (0.70-2.73)	0.501
	TT vs GT	0.80 (0.40-1.60)	0.535
Dominant Model	GG vs GT+TT	1.24 (0.69-2.22)	0.46
Recessive Model	TT vs GT+TT	0.89 (0.49-1.63)	0.718
	G/T	1.24 (0.84-1.84)	0.271

were determined as (31%) for the CC, (37%) for the heterozygous CT status, and (32%) for the TT, respectively.

Results showed that the genotype percentage among the patients and controls was the almost same, hence these groups were combined before statistical analysis (Table 2). Data showed that CC vs. CT and CC vs. CT+TT genotypes did not exhibited a significant difference between patients and controls (p=0.318) and the CC genotype was not associated with the disease [ORs, 1.51 (0.63-3.65)] (Table 2).

The ORs for the CC homozygote was [OR=1.23 (0.48-3.18); p=0.641] and that for the CT heterozygote was [OR=0.04 (0.74-5.68); p=0.127], and that for the TT homozygote [OR=1.66 (0.74-3.72); p=0.177] in each case. Frequency of T allele was 0.51 and 0.52 among patients and controls respectively and shows no association with breast cancer (OR, 1.06 (0.71-1.57); p=0.764).

In control women, the distribution of genotypes was found to be in Hardy-Weinberg Equilibrium (χ^2=0.17, p=0.95). There was no significant association between BRCA1 (rs799917) polymorphism and risk of breast cancer using a recessive or co-dominant model in the entire group (Table 3).

BRCA2 *(rs144848) polymorphism*

As shown in Table 2, the allele frequencies were determined as (38%) for the GG, (33%) for the heterozygous GT status, and (29%) for the TT, respectively.

The homozygous TT genotype was seen in lower percentage of patients with breast cancer (29%) when compared to healthy women (35%). Results showed that the genotype percentage among the patients and controls was the almost same, hence these groups were combined before statistical analysis (Table 2). Data showed that GG vs. GT genotype did not exhibited a significant difference between patients and controls (p=0.748). Similarly, GG vs. GT+TT and TT vs GT+TT genotypes exhibited no significant difference between patients and controls (p= 0.460 and 0.718 respectively) and were not associated with the disease 1.24 (0.69-2.22) and 0.89 (0.49-1.63) (Table 2).

The ORs for the GG homozygote was [OR=1.13 (0.70-2.73), p=0.501] and that for the GT heterozygote was [OR=1.11 (0.56-2.19), p=0.748], and that for the TT homozygote [OR=0.80 (0.40-1.60), p=0.535] in each case. Frequency of T allele was 0.45 and 0.51 among patients and controls respectively and showed no significantly association with breast cancer [OR=1.24 (0.84-1.84), p=0.271].

Discussion

To our knowledge, this is the first report of mutations in the *BRCA1* and *BRCA2* genes in Saudi breast cancer patients. Arabian populations have not been extensively studied; consequently knowledge of the prevalence and spectrum of BRCA1 and BRCA2 mutations in these populations is sparse. It has been estimated that about 1/3 to 2/3 of familial breast cancer are attributable to polymorphism in *BRCA1*, and about 10% of cases are attributable to polymorphism in *BRCA2* (Gayther et al., 1995; Spurdle et al., 2002; Reedy et al., 2002). Epidemiological data from Caucasian populations indicate that BRCA1 polymorphism account for 2-5% of all breast cancers and as much as 12% of early onset cases (Ford et al., 1998). The prevalence of *BRCA1* and *BRCA2* polymorphism in breast cancers in high-incidence, low- incidence and racially diverse populations has been well established. However, the information regarding the contribution of these genes in the incidence of and predisposition to breast cancer in ethnic Saudi families is lacking. Consanguinity is the choice and the risk factors associated with the breast cancer in other regions like the nulliparity, consumption of food are lacking. However, in the present study, nether *BRCA1* (rs799917) nor *BRCA2* (rs144848) polymorphisms showed any significant association with the predisposition of the disease (Table 2 and 3).

Recently in a Chinese study, *BRCA1* (rs799917) was found to be associated with breast cancer risk (Huo et al., 2009). Nicoloso et al. (2010) found that a codon SNP (rs799917) in BRCA1 gene, which is predicted to be a binding site for miR-638, was associated with increased risk of breast cancer and germline occurrence of *BRCA1* (rs799917) significantly varies among populations with different risks of developing breast cancer. Cox et al. (2005a) also have reported the association of *BRCA1* (rs799917) with breast cancer, but Sehl et al. (2009) showed a lack of association between the *BRCA1* (rs799917) and breast cancer. In spite of, several studies in different population for *BRCA1* (rs799917) which show association of *BRCA1* (rs799917) with breast cancer, our finding is in agreement with the finding of Sehl et al. (2009) because, present study failed to show any significant association of *BRCA1* (rs799917) with breast cancer.

Cox et al. (2005b) had reported a lack of association between *BRCA2* (rs144848) and the risk of breast cancer. Palli et al. (2007) suggested that the *BRCA2* TT homozygous genotype might be positively associated with an increased risk of MBC in men. There were no associations between *BRCA2* (rs144848) and overall risk of prostate cancer. Risks did not vary either by smoking or by family history of prostate cancer (Agalliu et al., 2010). Interestingly, association has been found between *BRCA2* (rs144848) and Chronic lymphocytic leukemia (Rudd et al., 2006). But the present study did not find any association between *BRCA2* (rs144848) and breast cancer.

Our study failed to show any association between *BRCA1* (rs799917) and *BRCA2* (rs144848) with the risk of breast cancer. Due to the low frequency of the patients reaching to the hospital, the number of samples was low in this study which was a limitation of the study.

In conclusion, Polymorphisms of breast cancer susceptibility gene *BRCA1* (rs799917) and *BRCA2* (rs144848) had no statistically significant correlation with clinico-pathological characteristics of breast cancer. Hence, we conclude that genetic polymorphisms in *BRCA1* (rs799917) and *BRCA2* (rs144848) could not play a role in the development of the breast cancers in the Saudi population. However a study with a larger sample number is before a firm conclusion about no role of these variants in breast cancer predisposition. In future, it will be interesting to explore if exposure to particular environmental carcinogens; different life style, reproductive pattern; dietary and cultural practices adopted by Saudi women could generate the variation in mutation pattern observed in present study.

Acknowledgements

The authors are thankful to the Deanship of Scientific Research at King Saud University, Saudi Arabia for funding this research through the Research Group Project No RGP-VPP-27.

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