Original Article
RAD51 (rs1801320) gene polymorphism and breast cancer risk in Turkish population

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Abstract: Background: Breast cancer is frequently observed multifactorial disorder worldwide and in Turkey as well. The genetic factors have been found as main cause of breast cancer development and the genes responsible for DNA repair have been shown to provide maintenance of chromosomal stability. RAD51 gene plays an important role in formation of Holliday junction which is branched intermediate product that occurs during homolog recombination-based DSB repair. RAD51 (rs1801320) gene variant is supposed to change RAD51 gene expression and contribute to DNA instability and development of cancer, including breast cancer. Objectives: In the light of these data, the purpose of this study is to investigate association between RAD51 (rs1801320) variant and breast cancer risk in hospital-based Turkish population. Materials and methods: Genotyping for RAD51 (rs1801320) variant in DNA samples of 548 breast cancer patients and 360 healthy women was done by PCR-RFLP method. SPSS software was used to calculate genotype and allele frequencies, p, χ^2 and OR (95% confidence intervals) values. Finally, Hardy-Weinberg equilibrium (HWE) was confirmed for tested patient and control populations and statistical power (SP) was calculated. Conclusion: This study showed no statistically significant association between RAD51 (rs1801320) variant and breast cancer (χ^2=0.852, P=0.653).

Keywords: Breast cancer, RAD51, rs1801320, Turkish population

Introduction

Breast cancer is widely encountered multifactorial disorder in women worldwide as well as women in Turkey and the second cause of death among women [http://www.cancer.org/cancer/breastcancer/detailedguide/breast-cancer-key-statistics, [1, 2]. Epidemiological studies on the determinants of breast cancer have indicated that environmental factors leading to double-strand breaks increase the breast cancer risk, whereas the genetic factors have been found as main cause of breast cancer development [3, 4]. Beside genetic alterations in the genes such as oncogene and tumor suppressor genes, the genes responsible for DNA repair in the case of double-strand breaks (DSB) or single-strand breaks (SSB) may lead to tumor formation [5, 6].

Homologous recombination (HR) is one of the double-strand break repair (DSBR) pathway in Eukaryotes and proteins having function in HR are important for appropriate DNA repair [7]. RAD51 protein, which is encoded by RAD51 gene located on 15q15.1 chromosomal location, plays a role in the formation of Holliday junction which is branched intermediate product that occurs during homolog recombination-based DSB repair (HRR) [8, 9]. In order to initiate HRR cutting of DNA ends and formation of 3' DNA overhangs for homology screening are required. On this basis, MRN complex is recruited to the DSB region and it supports cutting of DNA ends. At this time, some other proteins participate to achieve end-resection and formation of ssDNA which is then ssDNA coated with RPA proteins. In the following steps, RPA replaces with RAD51 and pre-synaptic RAD51 nucleofilament is composed. The resulting filament attacks to other dsDNA molecule in order to start homology screening. After the detection of homology, DNA synthesis begins at 3' end of attacking strand. At this stage, there are two possible choices for HRR; first is synthesis dependent strand annealing (SDSA) and the

second one is DSBR. As a result of these pathways, DSB repair is achieved and RAD51 proteins exhibit essential functions during the repair process [9].

Any alterations in normal function of RAD51 protein may cause hypersensitivity against radiation and induce low level mitotic and meiotic recombination [10]. Some studies has supported that even small changes occurred in RAD51 gene may lead to DNA instability and chromosomal deficiency which then cause carcinogenesis and malignancy due to the accumulation of genetic changes [11-13]. Also, RAD51 gene variations have been considered as a risk factor for breast cancer due to the altered RAD51 protein expression [12]. On the other hand, one study has shown that RAD51 and BRCA1 proteins were expressed in low levels in breast cancer cell lines and breast cancer cells. Similarly, the other study in which 30% of breast cancer patients have been shown to possess low-level RAD51 protein. As a result, RAD51 might involve in breast cancer tumorigenesis [14, 15].

RAD51 (rs1801320) variant (135G>C, substitution of G to C at position 135) is located in 5' untranslated region (5'UTR) of RAD51 gene. It has been supposed that this variant triggers aggressive tumor formation by influencing mRNA stability and/or translation efficiency of RAD51 gene which then leads to malfunctional RAD51 protein affecting DNA repair potential. Also it was correspondingly associated with RAD51 expression and DNA repair regulation. [11]. Although it has been suggested to affect cancer phenotype through the penetrance of BRCA1/2 mutations, its contribution to breast cancer is still being studied [10].

In the light of these data, the purpose of this study is to identify association between RAD51 (rs1801320) and breast cancer risk in hospital-based Turkish population and to investigate genotype and allele frequencies which may predispose to breast cancer.

Materials and methods

Subjects

The present study includes 548 female breast cancer patients [mean age ± Standard Deviation (SD): 50.92±12.33] and 360 healthy female controls [mean age ± SD: 49.95±8.83]. Patient group was consisted of sporadic breast cancer patients and recruited from General Surgery Departments in Faculty of Medicine of Marmara and Kocaeli University. The study was approved by ethical committee of the Kocaeli University and all subjects gave informed consent for participating in the study.

Genotyping

Genomic DNA was isolated from subjects based on conventional salting-out method [16]. Genotyping was done by using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Forward and reverse primers (10 pmol) for this variant were as follows: 5'-TGGGAACTGCAACTCATCTGG-3' and 5'-GCGCTCCTCTCTCCAGCA-3', respectively. PCR were performed by using following conditions; initial denaturation of genomic DNA 94°C for 3 min followed by 35 cycles at 95°C for 30 s, 53°C for 30 s, 72°C for 30 s and final extension with 72°C for 10 min. The obtained 157 bp PCR product was digested by 2 U MvaI restriction endonuclease that yields 86- and 71-bp at 37°C overnight. Thereafter polyacrylamide gel (8%) electrophoresis was performed for 35 min at 20 W and the resulting bands (157-bp for CC genotype, 157-, 86-, 71-bp for GC genotype and 86- and 71-bp for GG genotype) was visualized by silver staining procedure.

Statistical analysis

The Hardy-Weinberg equilibrium was verified by using the web tool available online (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl) for the breast cancer and control groups. The following statistical analyses were carried out by using SPSS software package, version 21.0 (IBM SPSS, Armonk, NY, USA). χ^2 test and Student's t-test were used to compare allele and genotype frequencies between case and control group. The odds ratio (OR) value was obtained in 95% confidence intervals (CI). *P* value less than 0.05 was accepted as statistically significant difference between groups. Eventually, statistical power (SP) of the study was designed in such a way that SP was above 0.80.

Results

Genotype and allele frequencies of RAD51 (rs1801320) variant were determined for 548

Table 1. RAD51 (rs1801320) genotype and allele frequencies in female breast cancer patients and control subjects

Gene	Cases, n (%)	Controls, n (%)	χ^2	P-value	OR (95% CI)*
RAD51 (rs1801320)	548 (100.0)	360 (100.0)	0.852	0.653	
GG	439 (80.1)	297 (82.5)	0.809	0.369	0.854 (0.606-1.204)
GC	103 (18.8)	60 (16.7)	0.669	0.414	1.157 (0.815-1.643)
CC	6 (1.1)	3 (0.8)	-	1.000	1.317 (0.327-5.301)
Allele frequency					
G	981 (90.0)	654 (91.0)	-	1.000	0.759 (0.189-3.005)
C	115 (10.0)	66 (9.0)	0.809	0.369	1.171 (0.830-1.650)
HWE exact (p)**	1.00	1.00			
SP***	1.00	1.00			

*OR, Odds ratio; CI, Confidence interval. **HWE, Hardy-Weinberg equilibrium. ***SP, Statistical power.

breast cancer patients and 360 control subjects. Both cases and controls were in HWE (P>0.05). The genotype frequencies for the RAD51 (rs1801320) variant were 80.1% GG, 18.8% GC and 1.1% CC in the cases and 82.5% GG, 16.7% GC and 0.8% CC in the control group. Allele frequencies of the G allele were 90.0% and 91.0% in the cases and controls, respectively. Allele frequencies of the C allele were 10.0% and 9.0% in the cases and controls, respectively. No statistically significant difference was found in genotype frequencies of the cases and controls (χ^2=0.852; P=0.653). Allele frequencies were also identified not to be statistically significant for G and C alleles (P=1.000 for the G allele, P=0.369 for C allele). SP value was found as 1.00 (**Table 1**).

Discussion

It has been known that breast cancer is mainly affected by genetic factors [3, 4]. RAD51 gene is one of the DNA repair gene playing several roles in DSBR and in maintenance of chromosomal stability [17]. In the present study, association between RAD51 (rs1801320) variant and breast cancer risk was studied in hospital-based Turkish female breast cancer patients and control subjects. Moreover, the genotype and allele frequencies for breast cancer were revealed.

The results suggested that there was not statistically significant association between RAD51 (rs1801320) variant and breast cancer risk (χ^2=0.852, P=0.653). Also, no statistical difference was found between both genotype and allele frequencies in cases and controls

(P>0.05 for genotype and allele frequencies) (**Table 1**).

Association studies on RAD51 (rs1801320) variant and breast cancer susceptibility were previously carried out in different countries and ethnicities. Studies performed in USA, Cyprus, Saudi Arabia and Turkey in which the association of RAD51 (rs1801320) variant and breast cancer risk are not statically significant are in agreement with our findings [13, 18-20]. However, several meta-analysis and studies in Iran, Poland have contradictory results which indicate statistically significant association [19, 21]. Moreover, the breast cancer predisposition of RAD51 (rs1801320) variant was analyzed among populations classified according to ethnicities and statistically significant protective effect was found for Asians but not for Caucasians [21]. The allele frequency results of this study are similar to that of Caucasians and RAD51 135 C allele frequency is greater in patients (C allele =10.0) as compared to controls (C allele =9.0) (**Table 1**). On the other hand, while 37.4% of black women have at least one copy of RAD51 135 C variant allele, the ratios alter as 15.9%, 9.6% and 17.3% in non-Jewish white women, Jewish white women and other ethnic groups, respectively [22]. In this study, the C allele frequency is similar to that of Jewish white women. Additionally, C allele frequency in breast cancer patients evolutionarily differs from one population to another: in a decreasing order; 30.28% in China, 15.33% in Poland, 14.57% in Russia, 12.72% in Korea, 11.77% in Poland, 10.00% in Turkey, 10.00% in Portugal, 8.10% in Australia, 6.93% in Chile, 6.47% in Germany (**Table 2**).

Table 2. Genotype distribution and C allele frequency of RAD51 (rs1801320) variant in different breast cancer populations

Populations	Total number	GG	GC	CC	C allele frequency
China [23]	71	35	29	7	43 (30.28)
Poland [24]	150	108	38	4	46 (15.33)
Russia [25]	151	111	36	4	44 (14.57)
Korea [26]	782	611	143	28	199 (12.72)
Poland [27]	1007	785	207	15	237 (11.77)
Turkey (This study)	548	439	103	6	115 (10.00)
Portugal [28]	265	216	45	4	53 (10.00)
Australia [29]	1444	1221	212	11	234 (8.10)
Chile [30]	267	232	33	2	37 (6.93)
Germany [31]	2172	1904	255	13	281 (6.47)

As seen above, different studies and ethnic origins may give rise to dissimilar results for the RAD51 (rs1801320) variant distribution. Since the cancer is multifactorial disorder, different genetic background of various ethnic groups may cause this inconsistent result. For instance, the effect of RAD51 135 C allele can be suppressed by other genes involved in the breast cancer development in Caucasians and therefore it may not provide the same effect with Asians [21]. In addition, environmental factors such geographical locations and life styles may influence differences between populations.

In Turkey, only one study including 147 familial breast cancer patients and 120 control subjects has been performed on the association of RAD51 (rs1801320) variant and breast cancer risk. Allele and genotype frequencies were found as significantly different between cases and controls [20]. The significant difference obtained by Akisik et al. was inconsistent with our results. This inconsistency might arise from different population size, BRCA1/2 status and breast cancer type which was sporadic in this study and familial in the study performed by Akisik et al. On the other hand, the results of these studies were in agreement in terms of more C allele frequencies in the patient groups.

Our study demonstrated the allele and genotype frequencies of RAD51 (rs1801320) variant for the largest Turkish breast cancer patient population to date. Additionally, HWE (1.00) and SP (1.00) values indicated that study populations were in balance and the obtained results for this study were reliable. In contrast to these strengths, there are also some limitations. Even if control population consists of healthy women, individuals might be at the onset of disorder and therefore they did not show symptoms of the disease. In addition, having personal information (including menopause age, education level, body mass index and menarche age) and clinical information (including estrogen and progesterone receptor level, tumor grade) of individuals may allow more detailed results for association.

In future, existing limitations can be overwhelmed and RAD51 (rs1801320) variant may be studied in well-defined population in the presence of clinical information. In addition, besides the different polymorphisms of RAD51, other genes responsible for DSB such as BLM, TP53, PTEN, ATM, NBN, XRCC2/3 can be analyzed to show allele and genotype frequencies for Turkish population. This may be an important step to display the mechanism of the disease. To show combined effects of polymorphisms on breast cancer risk and to obtain prior information about their breast cancer susceptibility, haplotype analysis can be performed for RAD51 and other repair genes. Moreover, functional effects of RAD51 (rs1801320) variant may be clarified with mRNA and protein studies. Eventually, gene-gene and gene-environment interactions can be focus of the studies in order to understand better multifactorial nature of cancer. Based on this data, genotype-specific drugs can be designed and used for treatment. Finally, decrease in the disease progression and increase in the yield of treatment may be achieved.

Disclosure of conflict of interest

None.

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