Breast Cancer (2018) 25:350–355
https://doi.org/10.1007/s12282-018-0837-9

ORIGINAL ARTICLE

Breast cancer risk associated with genes encoding DNA repair MRN complex: a study from Punjab, Pakistan

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Received: 5 September 2017 / Accepted: 18 January 2018 / Published online: 24 January 2018

Abstract

Background Variants of DNA repair genes are extensively reported to cause genetic instability and increase the risk of breast cancer. In combination with *NBS1*, *MRE11* and *RAD50* constitute an MRN (MRE11–RAD50–NBS1) complex that repairs DNA damage. However, certain genetic alterations in *MRE11* and *RAD50* produce abnormal protein that affects the repairing process and may result in malignancy. We aimed to investigate the association of *MRE11* and *RAD50* polymorphisms with breast risk in the female population of Punjab, Pakistan.

Methods We collected blood samples of 100 breast cancer patients and 100 tumor-free females selected as controls. Extracted DNA was genotyped by tetra ARMS-PCR followed by gel electrophoresis. Results were analyzed by SPSS and SNPstats to analyze the association of different clinical factors and SNPs (single nucleotide polymorphisms) with the risk of breast cancer.

Results We found that the increased risk of breast cancer is associated with *MRE11* variant rs684507 (odds ratio-OR 3.71, 95% confidence interval-CI 1.68–8.18, p value < 0.0001), whereas, *RAD50* variant rs28903089 appeared to have protective effect (OR 0.55, CI 0.29–1.02, p value $= 0.003$). Additionally, clinical factors such as positive family history, life style, and marital status also play significant roles in breast cancer development.

Conclusion In the present study, strong risk of breast cancer was associated with *MRE11* gene. However, *RAD50* showed protective effect. Additionally, clinical factors are also pivotal in risk assessment. We anticipate that targeting specific genetic variations confined to ethnic groups would be more effective in future therapeutic approaches for prevention and treatment of breast cancer.

Keywords Breast cancer · *RAD50* · *MRE11* · Genetic variants · Pakistan

Introduction

Breast cancer is one of the predominant malignancies worldwide, which is characterized by uncontrolled proliferation of breast cells [1–3]. According to an estimate, the breast cancer prevalence is increasing by the rate of 2% each year [4]; however in Pakistan, the incidence rate is 1/9 that is higher than other Asian countries [5]. Although, breast cancer is very common in females but it can occur in male population as well [6, 7]. Several explanatory factors such as age, gender and family history are involved in cancer development [5, 8]. It is well established that the women older than 40 years are more susceptible to breast cancer. Moreover, positive family history also plays a vital role in disease prevalence. Other responsible factors may include genetic alteration, hormonal imbalance, exposure to chemicals, UV radiations, life style, and certain bacteria and viruses [9, 10]. Genetic association in breast cancer has been reported in several studies and suggests that the penetrant genes increase the familial risk of early onset cancer [11]. Mutation in certain genes that participate in cell proliferation, DNA double-strand break repair (DSBR) pathway and activation of cell cycle checkpoint may lead to cancer development [12–14].

The MRN complex, comprising of proteins encoded by the genes *MRE11*, *RAD50*, and *NBS1,* plays a vital role in DNA DSBR pathway by recruiting Ataxia telangiectasia mutated (ATM) to damaged sites that activates DNA repair

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network. Several studies have suggested that the carriers of ATM mutations and polymorphic variants are at higher risk of breast cancer. Principally, the ATM protein is presumed to be a chief activator of DNA DSBR network, and becomes activated only after recruitment to DSB (double-strand break) sites [15].

Among the MRN complex genes, *MRE11* encodes specific proteins that are involved in maintenance of telomere length, DSBR pathway and homologous recombination [15]. Another gene of MRN complex "RAD50" is required to keep DNA in close proximity by binding its ends. This gene is responsible to produce a protein that is essential in DNA DSBR pathway [16].

The structure–function studies of the MRN complex suggested that *MRE11* subunit is necessary for the aphidicolin (APH)-induced activation of Chk1 [17]. Thomson et al. [18] reported that the MRN complex, particularly the nuclease activity of *MRE11*, plays a significant role in the activation of Chk1 in response to stalled replication forks. When cells cope with DNA lesions, Chk1 acts as the workhorse of checkpoint response by delaying the cell cycle and controlling the replication apparatus. *RAD50* is a member of the ATP-binding cassette family of ATPases and contains coiled-coil domains characteristic of structural maintenance of chromosomes (SMC) proteins. *RAD50* possesses both ATPase and adenylate kinase activities. Overall, *RAD50* is important for the conformational dynamics of the MRN complex in response to various DNA structures. Lee and Dunphy [17] reported that MRE11–RAD50 (MR) complex without *NBS1* was able to support phosphorylation of Chk1 as efficiently as the complete MRN complex. Hence, *NBS1* appears to be dispensable for the APH-induced activation of Chk1 under these conditions.

It has been well established that the single nucleotide polymorphisms (SNPs) are most frequent genetic variations which may result in harmful effects on an individual [19–21]. Therefore, certain SNPs in *MRE11* and *RAD50* genes are strongly associated with cancer development and could be useful in diagnosis and treatment [22]. In contrast, several studies reported no association of *NBS1* with the risk of several malignancies including breast cancer [23–25]. Therefore, present study was designed to evaluate the role of *MRE11* and *RAD50* genes in breast cancer development among the female population of Punjab, Pakistan. For this purpose, we conducted a case–control study to estimate the possible association of rs684507 (*MRE11*) and rs28903089 (*RAD50*) with breast cancer incidence.

Materials and methods

Study population

A total of 200 subjects with same age and sex group, including breast cancer patients and healthy individuals were selected. Briefly, 100 patients from different selected hospitals were recruited for blood samples and required information. Inclusion criteria for breast cancer subjects include (1) women with well-diagnosed breast cancer and (2) the parents of selected patients should be of Pakistani origin. The patients with any infectious disease were excluded from the study. The background of patients recruited in present study is given in Table 1. Sampling was carried out during the period of July 2015 to February 2016. The control subjects were taken from general population with normal health status. All the subjects were genetically unrelated and their blood samples were drawn with their consent. An ethical approval was obtained from Ethical Board of Institution to conduct study on human population.

Table 1 Distribution of different stratification factors among patients

Sr. no	Variables	No. of patients
1.	Marital status	
	Married	90
	Unmarried	10
2.	Life style	
	Housewife	83
	Working	17
3.	Family history	
	Yes	23
	No	77
4.	Disease status	
	Bilateral	11
	Unilateral right	39
	Unilateral left	50
5.	Metastatic status	
	No metastasis	58
	Metastasis	42
6.	Treatment type	
	Chemotherapy	48
	Radiotherapy	19
	Chemotherapy + surgery	10
	Radiotherapy + surgery	14
	Surgery	5
	Chemotherapy + HRT	4

Blood sampling

A 2–3 mL of blood was drawn from median cubital vein of the patient under aseptic conditions and stored in ethylenediaminetetraacetic (EDTA) vials at − 20 °C until proceeded for DNA isolation.

Genotyping

Isolation of genomic DNA was done using modified chloroform; isoamylalcohol protocol followed by gel electrophoresis. Samples were diluted and quantified to obtain the required DNA concentration of 10 ng/µL. Frequently reported SNPs (i.e., rs684507; *MRE11* and rs28903089; *RAD50*) associated with breast cancer were selected for further study. Genotyping was carried out by Tetra-ARMS PCR following the protocol described earlier [26] with minor modifications in the quantity of chemicals that are given in Table 2. The primer sequences used in the study are given below.

MRE11 (rs684507):

Forward inner primer 5′-AGAACCGTATGTGACCCTTTCTGACT

Reverse inner primer 5′-ATGGGAAATTAATGTATGCTAAATAACTG

Forward outer primer 5′-CGTGTTTGTTTATTTACACTCGCTTTAA

Reverse outer primer 5′-GGCAAATTTAGAAGTCTCATTTTCATCT

RAD50 (rs28903089):

Forward inner primer 5′-TCGTGATCAGATTACAAGTAAGGAGGA

Reverse inner primer 5′-TGACAATTTCCTTTGAAGATGTTAACTAGG

Forward outer primer 5′-AGTGACAGCATAATATCCCACTGTATGAA

Reverse outer primer 5′-ACGTTAAATAGCTTGATTTAGCCAGTCC

For *MRE11* SNP, PCR program was optimized as follows:

Table 2 Components and concentrations of tetra-ARMS PCR reaction mixture

Sr. no	Components	Amounts (µL)
1.	Master mix (2X)	12.5
2.	Nuclease-free H_2O	6.0
3.	Forward inner primer	1.0
4.	Reverse inner primer	1.0
5.	Forward outer primer (5′–3′)	1.0
6.	Reverse outer primer (5′–3′)	1.0
7.	DNA	2.0
8.	Taq polymerase	0.5

An initial denaturation at 94 °C for 4 min, followed by 35 cycles—denaturation at 94 °C for 1 min, annealing at 59 °C for 1.5 min, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min.

For *RAD0* SNP, PCR program was optimized at an initial denaturation at 94 °C for 4 min, followed by 35 cycles—denaturation at 94 °C for 1 min, annealing at 60 °C for 1.5 min, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. Subsequently, PCR products were checked on 2% agarose gel.

Statistical analyses

Chi-square test of association was done to analyze the association of different clinical factors with the risk of breast cancer in patients in comparison with controls using SPSS (Version 22). Exact test for Hardy–Weinberg equilibrium was applied for both genetic variants. Allelic and genotypic frequencies were calculated using SNPStats.

Results

We found that breast cancer is significantly associated with positive family history, married women and house wives in patients compared to controls (Table 3). In the present study, two SNPs were genotyped by tetra-ARMS PCR to reveal their association with breast cancer. The *MRE11* SNP showed significant association with cancer development (Exact test for Hardy–Weinberg equilibrium, p value < 0.0001; Table 4). Detailed analysis showed that the individual alleles are not significantly associated with disease development (OR 1.27, CI 0.69–2.36, p value = 0.43) as the proportion of variant C is almost same in both groups (i.e., patients and controls). However, the genotype frequency showed significant association with breast cancer (OR 3.71, CI 1.68–8.18, p value < 0.00001; Table 5) and can cause more than three times higher risk for disease development. In detail, *MRE11* SNP showed overdominant model (logistic regression model, p value < 0.0001) and confirmed that the heterozygous TC

Table 3 Test of association for clinical factors

Parameters	Patients	Controls	Chi-square	p value
Positive family history	23	10	6.13	0.03*
Negative family history	77	90		
Married	90	75	5.1	0.02*
Unmarried	10	21		
House wife	83	65	13.26	0.0002*
Working	17	35		

*Significant association

Table 4 Exact test for Hardy–Weinberg equilibrium ($n = 200$)

Subjects	*MRE11* rs684507				*RAD50* rs28903089			
	TT (%)	CC (%)	TC (%)	*p* value	AA (%)	CC (%)	AC (%)	*p* value
Controls	57	10	33	0.13	53	11	36	0.208
Patients	64	26	10	< 0.0001*	33	26	41	0.07

*Significant association

Table 5 Allelic and genotypic frequency in study subjects

Allele/genotype	Case (%)	Control (%)	Odds ratio	Confidence interval 95%	*p* value
MRE11 (rs684507)					
T	69	74	1.28	(0.6907–2.3672)	0.4340
C	31	26			
TT	64	57	1.00		< 0.00001*
CC	26	10	3.71	(1.68–8.18)	
TC	10	33	0.43	(0.19–0.97)	
RAD50 (rs28903089)					
A	53	73	2.40	(1.3279–4.3290)	0.0037*
C	47	27			
AA	33	53	1.00		0.0036*
CC	26	11	0.55	(0.29–1.02)	
AC	41	36	0.26	(0.12–0.60)	

CI confidence interval

*Significant association

Table 6 *MRE11* genetic variant association with response subject (crude analysis $n = 200$)

Model	Genotype	Controls	Patients	OR (95% CI)	*p* value	AIC
Dominant	T/T	57 (57%)	64 (64%)	1.00	0.31	280.2
	T/C–C/C	43 (43%)	36 (36%)	1.34 (0.76–2.37)		
Recessive	T/T–T/C	90 (90%)	74 (74%)	1.00	0.0028	272.3
	C/C	10 (10%)	26 (26%)	0.32 (0.14–0.70)		
Overdominant	T/T–C/C	67 (67%)	90 (90%)	1.00	1.00E−04*	264.9[a]
	T/C	33 (33%)	10 (10%)	4.43 (2.04–9.62)		

*Significant association

[a]Best proposed model

was more responsible for disease compared to homozygotes (TT, CC; see Table 6).

The SNP rs28903089 of *RAD50* showed a slight association with the disease (Exact test for Hardy–Weinberg equilibrium, *p* value 0.07; Table 4). Allelic frequencies showed significant association of variant with breast cancer (OR 2.3976, CI 1.32–4.32, $p = 0.0037$). The genotype frequencies also showed significant association with disease (OR 0.55, CI 0.29–1.02, $p = 0.0036$) acting as a protective allele.

As the result of regression analysis, single copy of variant *C* was mainly responsible (logistic regression, *p* value < 0.005) to decrease the risk of disease whereas heterozygous (AC) and homozygous genotypes had the same effect (Table 7).

Discussion

Breast cancer is the most frequently diagnosed malignancy and second leading cause of cancer death among women worldwide. Beside alterations in certain genes, several factors such as family history, marital status and lifestyle play significant roles in cancer development [27]. In the present study, we found that the risk of breast cancer is associated with patients that have positive family history, married and house wives compared to controls (Table 3). Due to the importance of MRN complex genes in maintenance of the genomic integrity and DNA damage repair to prevent malignancy, we analyzed the significance of two MRN complex polymorphisms for the risk of breast cancer. Both of the SNPs showed significant association with malignancy of

Table 7 *RAD50* genetic variant association with response subject (crude analysis $n = 200$)

Model	Genotype	Controls	Patients	OR (95% CI)	p value	AIC
Dominant	A/A	53 (53%)	33 (33%)	1.00	0.0041*	273[a]
	A/C–C/C	47 (47%)	67 (67%)	0.44 (0.25–0.77)		
Recessive	A/A–A/C	89 (89%)	74 (74%)	1.00	0.0057	273.6
	C/C	11 (11%)	26 (26%)	0.35 (0.16–0.76)		
Overdominant	A/A–C/C	64 (64%)	59 (59%)	1.00	0.47	280.7
	A/C	36 (36%)	41 (41%)	0.81 (0.46–1.43)		

*Significant association

[a]Best proposed model

breast tissues. The polymorphisms of *RAD50* and *MRE11* have been widely associated with cancer as they are among the major DNA damage repair genes [28].

We observed that the *MRE11* SNP showed significant association with elevated risk of breast cancer and the heterozygous TC was mainly responsible for disease (OR 3.71, CI 1.68–8.18, p value < 0.00001). In contrast, the SNP rs28903089 of *RAD50* showed a slight association with the disease and single copy of variant C is giving protective effect against cancer development (OR 0.55, CI 0.29–1.02, $p = 0.0036$). It has been well established that variations in DNA damage repair genes are highly associated with increased risk of breast cancer [29]. Basically, alteration in genes of DNA DSBR pathway affects DNA double-strand repair and maintenance of telomere length [3, 30–32]. Dysregulation of these pathways may lead to cancer development. Several studies have reported the involvement of *RAD50* and *MRE11* SNPs in breast cancer, nevertheless variation may occur among different populations [33]. Kuschel et al. [34] and Heikkinen et al. [33] reported a strong association of *MRE11* and *RAD50* SNPs with breast cancer in specific populations.

In contrast, our results showed no significant association of *RAD50* SNP rs28903089 with increased risk of breast cancer. It might be due to the fact that the *RAD50* SNP rs28903089 is rare in breast cancer and have low penetrance. Furthermore, its association could not be detected because of limited sample size. Similar results have been reported elsewhere [35, 36]. These data indicate that *RAD50* mutations are rare in familial breast cancer and either carry no, or a very small, increased risk of cancer. Altogether, these results suggest *RAD50* can only be making a very minor contribution to familial breast cancer predisposition. *MRE11* and *RAD50* polymorphisms are well studied in different populations but their association patterns are controversial [37]. Some studies report these SNPs playing protective role while other associate them with increased risk of disease [6, 38–40].

More comprehensive studies with larger sample size are required to determine broader range of different factors involved in breast cancer. These studies will aim to eradicate the limitations of present study and also to validate its results and findings. Despite of having some limitations, the findings of the present study are remarkable. Both markers showed association with the onset of breast cancer. Due to the findings of this research, the markers which are studied here can open up ways to improve the present treatment strategies against breast cancer in upcoming future.

Conclusion

In conclusion, our results suggest that not every polymorphism is associated with the increased risk of disease as in the case of *RAD50* SNP but the SNP of *MRE11* is involved in increased risk of breast cancer development as it plays a significant role in DNA damage repair. Both SNPs, more or less, are associated with response subjects in the studied population. Major proportion of patients was heterozygous showing the effectiveness of variants in disease population with dominant and over dominant model.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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