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Original Article

An association between single nucleotide polymorphisms (SNPs) of -4719A/T (rs2619679) and -4601A/G (rs5030789) RAD51 gene and breast cancer

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Abstract: The double-strand break DNA repair pathway, including RAD51 gene, is implicated in maintaining genomic stability and therefore could affect breast cancer risk. The purpose of this study was to evaluate the clinical significance of the RAD51 gene polymorphisms in patients with breast cancer. The study included 1200 patients: 600 with breast cancer and 600 healthy controls. The -4719A/T (rs2619679) and the -4601A/G (rs5030789) RAD51 gene polymorphisms have been studied in DNA isolated from blood samples. The associations of the analysed genotypes and clinical data at diagnosis have been evaluated. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each genotype and allele. In the present study, an association was identified between RAD51 -4719A/T and the -4601A/G polymorphisms and the incidence of breast cancer. A relationship was confirmed between RAD51 polymorphisms and breast cancer progression, assessed by histological stages. This is the first study, linking -4719A/T and the -4601A/G RAD51 gene polymorphisms with breast cancer development. In conclusion, RAD51 polymorphisms may be regarded as predictive factors of breast cancer in female population.

Keywords: Breast cancer, *RAD51*, rs2619679, rs5030789, polymorphism

Introduction

Double-strand DNA breaks (DSBs) are the most dangerous DNA damage. If not repaired, they cause loss of chromosomes and cell death. An accumulation of DSBs destabilizes the genome and rearranges it, leading to down-regulation of transcription and development of various diseases (Jackson, 2002). DSBs are repaired by the following two mechanisms: homologous recombination (HR) and non-homologous end joining (NHEJ) [1].

RAD51 homolog (RecA homolog, E. coli) (*S. cerevisiae*) is involved in the homologous recombination and repair of double-strand breaks in DNA and DNA cross-links, as well as in the maintenance of chromosome stability [2]. Literature data suggest that RAD51 levels do not generally increase in normal cells [3].

Raderschall et al. [4] showed that increased levels of RAD51 in tumour cells were found to be associated with unscheduled HR and genetic instability. Therefore, the elevated levels of RAD51 may be signalling the presence of extensive DNA damage. Changes in RAD51 biosynthesis are usually preceded by changes in its gene transcription and mRNA level. Gene variability could contribute to the level of RAD51 biosynthesis [5].

The *RAD51* gene has been mapped to 15q14-15 chromosome and is highly polymorphic in nature. A G to C substitution at position 135 and G to T substitution at position 172 of the *RAD51* gene (5'-untranslated region) have been described as single nucleotide polymorphisms (SNPs). Both polymorphisms are located in the regulatory element of the *RAD51* promoter and are suggested to be associated with messenger RNA stability and expression [5, 6].

Some reports provide proof that the *RAD51* G135C (rs1801320) and G172T (rs1801321) polymorphism were related to increased risk of various cancers [7-12].

Table 1. The characteristic of patients (n = 600) and controls (n = 600)

Characteristics	Breast cancer patients (number/%)	Controls (number/%)
Menarche (years)		
10	130 (22%)	123 (22%)
11	138 (23%)	121 (20%)
12	139 (23%)	116 (19%)
13	78 (13%)	85 (14%)
14	70 (12%)	82 (13%)
≥ 15	45 (7%)	73 (12%)
Parity		
Nulliparous	150 (25%)	146 (24%)
1	141 (24%)	146 (24%)
2	142 (24%)	138 (22%)
3	85 (14%)	90 (15%)
≥ 4	82 (13%)	80 (14%)
Menopause status		
Premenopausal	295 (49%)	289 (48%)
Postmenopausal	305 (51%)	311 (52%)
Use of menopausal hormones		
Never	202 (34%)	295 (49%)
Estrogen	398 (66%)	305 (51%)
Bloom-Richardson grading		
I	200 (33%)	
II	350 (59%)	
III	50 (8%)	
Tumor size grade		
T1	57 (10%)	
T2	203 (34%)	
T3	340 (56%)	
Lymph node status		
N0	330 (55%)	
N1	148 (25%)	
N2	76 (13%)	
N3	46 (7%)	

We supposed that other genetic variability could act additively or independently to 5'UTR polymorphism what might clarify the attitude of *RAD51* in tumors progression.

Mucha et al. [13] examined the role of -4719A/T (rs2619679) and -4601A/G (rs5030789) SNPs in *RAD51* gene and risk of colorectal cancer. The presence of the *RAD51* G/A genotype was associated with the increased risk of cancer progression in patients. Yet, to our knowledge, there are no reports that assess the effect of this genetic alteration on the risk of breast cancer. Aim of this study was to analyze the frequency of alleles and genotypes of SNPs -4719A/T (rs2619679) and -4601A/G (rs5030789) in *RAD51* and an attempt to determine the impact this polymorphism exerts on breast cancer.

Patients and methods

Patients

All patients and healthy subjects were Caucasian. We enrolled only women born and living in central Poland (Łódź region). In the reported study, blood samples were collected from 600 women with ductal breast carcinoma, treated at the Department of Oncology, Institute of Polish Mother's Memorial Hospital, Lodz, Poland. The age of the patients ranged in from 42 to 82 years (the mean age 49.2 ± 11.12). No distant metastases were found in any of the patients at the time of treatment onset. The average tumor size was 20 mm (the range 17-32 mm). All the tumors were graded by a method, based on the criteria of Scarf-Bloom-Richardson. The demographic data and the pathologic features of the patients are summarized in **Table 1**. Blood samples from age-matched, cancer-free women (n = 600) served as control (the mean age 48.43 ± 19.21). An appropriate ethical approval was obtained from the Ethics Committee of the Institute of Polish Mother's Memorial Hospital, Lodz, Poland.

DNA was extracted from the material, using a commercially available DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instruction. Analysis of the -4719A/T (rs2619679) and -4601A/G (rs5030789) polymorphism was performer by help of Light Cycler® 480 High Resolution Melting Master Kit (Roche, Mannheim, Germany) according to recommendations of producer. PCR amplification was performed in LightCycler® 96 (Roche, Mannheim, Germany) Thermocycler. Collected data were analyzed using LightCycler® 96 software version SW 1.1 (Roche, Mannheim, Germany).

Statistical analysis

Genotype frequency deviations were assessed for each polymorphism, comparing Hardy-Wein-

Table 2. Distribution of genotypes and alleles and odds ratios (OR) of the -4719A/T and -4601A/G polymorphism of the RAD51 gene in patients with breast cancer and controls

	Patients (n = 600)		Controls (n = 600)			
-4719A/T	Number	(%)	Number	(%)	OR (95% CI)[a]	*P*[b]
A/A	69	12	132	22	1.00 Ref	
A/T	72	12	360	60	0.38 (0.26-0.56)	< 0.001
T/T	459	76	108	18	8.13 (5.67-11.63)	< 0.001
A	210	18	624	52	1.00 Ref	
T	990	82	576	48	5.10 (4.23-6.16)	< 0.001
-4601A/G	Number	(%)	Number	(%)	OR (95% CI)[a]	*P*
A/A	108	18	192	32	1.00 Ref	
A/G	132	22	240	40	0.97 (0.71-1.34)	1.000
G/G	360	60	168	28	4.64 (3.41-6.30)	< 0.001
A	348	29	624	52	1.00 Ref	
G	852	71	576	48	2.65 (2.24-3.13)	< 0.001

[a]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [b]Chi square.

Table 3. Dependence of genotypes and frequencies of RAD51 gene polymorphisms alleles on tumour grade in patients with breast cancer[a]

	Breast cancer patients			
Grade[b]	I (n = 200)	II + III (n = 400)	OR (95% CI)[c]	*P*[d]
RAD51 -4719A/T	Number (%)	Number (%)		
A/A	30 (15)	39 (10)	1.00 Ref	
A/T	28 (14)	44 (11)	0.83 (0.42-1.61)	0.699
T/T	142 (71)	317 (79)	0.58 (0.34-0.98)	0.053
A	88 (22)	122 (15)	1.00 Ref	
T	312 (78)	678 (85)	0.63 (0.47-0.87)	0.005
RAD51 -4601A/G				
A/A	40 (20)	68 (17)	1.00 Ref	
A/G	72 (36)	60 (15)	2.04 (1.21-3.42)	0.010
G/G	88 (44)	272 (68)	0.55 (0.35-0.87)	0.014
A	152 (38)	196 (25)	1.00 Ref	
G	248 (62)	604 (75)	0.53 (0.41-0.69)	<0.001

Data in boldface are statistically significant. [a]n = 600; [b]according to Scarf-Bloom-Richardson criteria; [c]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [d]Chi square.

berg equilibrium values with control values by the standard Chi-square test. Genotype frequencies in the study cases and the controls were compared by the Chi-square test. Genotype specific risks were estimated as odds ratios (ORs) with associated 95% intervals (CIs) by unconditional logistic regression. *P*-values < 0.05 were considered significant. All the statistical analyses were performed, using the STATISTICA 6.0 software (Statsoft, Tulsa, Oklahoma, USA).

Results

Table 2 shows genotype distribution values of *RAD51* -4719A/T and -4601A/G polymorphisms in breast cancer patients and controls. In the present work we succeeded to demonstrate that T/T genotype of -4719A/T polymorphism of *RAD51* gene was associated with an increased risk of breast cancer in studied population, almost eight (OR 8.13; 95% CI 5.67-11.63, P < 0.001) times higher than in case of the other genotypes. We observed that T allele of -4719A/T polymorphism was strongly associated with breast cancer (OR 5.10; 95% CI 4.23-6.16, P < 0.001).

The second studied polymorphism of the gene, namely -4601A/G, was associated with the occurrence of breast cancer. We showed that G/G genotype of -4601A/G polymorphism of *RAD51* gene was strongly associated with the incidence of the tumour (OR 4.64; 95% CI 3.41-6.30, P < 0.001). We have demonstrated in the presented report that the G variant may increase the risk of studied tumour occurrence (OR 2.65; 95% CI 2.24-3.13, P < 0.001).

Histological grading was related to *RAD51* -4719A/T and -4601A/G polymorphisms (**Table 3**). Some correlation was observed between the genotypes of *RAD51* polymorphisms and breast cancer invasiveness. A statistically significant increase was observed, regarding A/G heterozygotes frequency (OR 2.04; 95% CI 1.21-3.42, P = 0.010) in grade I patients, according to Scarf-Bloom-Richardson classification.

However the current study failed to show the correlation between analysed genes polymorphisms and tumor size (T) and lymph node status (N) (**Table 4**). DNA repair genes polymor-

Table 4. RAD51 gene polymorphism and breast cancer progression[a]

	Breast cancer patients (n = 600)			Breast cancer patients (n = 600)		
	Tumor size			Node status		
	T1+T2 N = 260	T3 N = 340	OR (95% CI)[a]	N+ (n = 270)	N- (n = 330)	OR (95% CI)[b]
-4719A/T	Number (%)	Number (%)		Number (%)	Number (%)	
A/A	28 (11)	41 (12)	1.00 Ref	26 (10)	43 (13)	1.00 Ref
A/T	32 (12)	40 (12)	1.17 (0.60-2.29)	33 (12)	39 (12)	1.39 (0.71-2.74)
T/T	200 (77)	259 (76)	1.13 (0.68-1.89)	211 (78)	248 (75)	1.41 (0.83-2.37)
A	88 (17)	122 (18)	1.00 Ref	85 (16)	125 (19)	1.00 Ref
T	432 (83)	558 (82)	1.07 (0.79-1.45)	455 (84)	535 (81)	1.25 (0.92-1.69)
-4601A/G						
A/A	48 (18)	60 (18)	1.00 Ref	50 (19)	58 (18)	1.00 Ref
A/G	60 (23)	72 (21)	1.04 (0.62-1.73)	58 (21)	74 (22)	0.91 (0.54-1.52)
G/G	152 (58)	208 (61)	0.91 (0.59-1.41)	162 (60)	198 (60)	0.95 (0.61-1.46)
A	156 (30)	192 (28)	1.00 Ref	158 (29)	190 (29)	1.00 Ref
G	364 (70)	488 (72)	0.92 (0.71-1.18)	382 (71)	470 (71)	0.97 (0.76-1.26)

[a]T3 vs. T1+T2, [b]N - (node negative) vs. N + (node positive).

phisms were also unrelated to the patient's age, menarche, parity, menopause status and family history of cancer ($P > 0.05$).

Discussion

In the presented study, the role of polymorphisms was studied in DNA DSB repair *RAD51* gene, the polymorphisms being regarded as risk factors for breast cancer in a case setting. The following SNPs were considered in the homologous recombination pathway: *RAD51* -4719A/T (rs2619679) and -4601A/G (rs50-30789). This is the first study which has analysed the incidence of alleles of the *RAD51* -4601A/G and -4719A/T polymorphisms in samples from patients with breast cancer.

Homologous recombination repair plays a critical role in repairing DNA damage. The RAD51 protein is a core component of DNA double strand break repair by HR. The cells, which are deficient in this gene product, are defective in homologous recombination and demonstrate genomic instability [6].

The *RAD51* polymorphisms were found to be associated with various cancer diseases [8, 10, 13-15]. In our earlier studies we investigated the single nucleotide polymorphisms G135C and G172T in *RAD51* gene in breast, ovarian, endometrial, laryngeal and colorectal carcinoma [11, 16-20]. We found that the SNPs of the *RAD51* gene may increase the risk of investigated cancers.

Presented paper is a continuation of our screening research on SNPs within genes encodes protein participating in the repair of DNA DSBs. Therefore, we analysed the role of -4601A/G and -4719A/T genetic variations in the homologous recombination repair *RAD51* gene and in the risk of breast cancer. The effect of *RAD51* -4601A/G and -4719A/T polymorphisms on breast cancer occurrence was not investigated before.

In the studies on a series of 600 DNA samples from patients with breast cancer, originating from an ethnically homogenous population, we found a relationship of the studied polymorphisms with breast cancer occurrence. We demonstrated that T allele of -4719A/T polymorphism of *RAD51* gene was strongly associated with breast cancer. The *RAD51*-T/T genotype was connected with a higher risk for the tumour formation than it was in the case of the other genotypes. There was a 8.13-fold increased risk of breast cancer for *RAD51*-T/T genotype carriers, compared with subjects with *RAD51*-A/T, *RAD51*-A/A genotype, respectively. An association was observed between breast carcinoma occurrence and the presence of G/G genotype of 4601A/G polymorphism. Variant G allele of *RAD51* increased cancer risk. Some

correlation was observed between the *RAD51* -4601A/G and *RAD51* -4719A/T genotype and breast cancer invasiveness. A strong increase was observed, regarding A/G heterozygotes frequency in grade I patients. We did not find any association of the *RAD51* 4601A/G and 4719A/T polymorphisms in patients with cancer progression assessed by tumor size and node status.

In conclusion, the presented study implies that -4601A/G and -4719A/T polymorphisms of the *RAD51* gene may be associated with breast cancer. It appears from a thorough review of the medical literature that, the polymorphisms in *RAD51* gene, involved in the DNA repair pathway, have for the first time been analyzed in breast cancer patients.

Disclosure of conflict of interest

None.

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