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RESEARCH

BLM and *RAD51* Genes Polymorphism and Susceptibility to Breast Cancer

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Abstract DNA repair by homologous recombination is one of the main processes of DNA double strand breaks repair. In the present work we performed a case-control study (304 cases and 319 controls) to check an association between the genotypes of the c.-61 G>T and the g.38922 C>G polymorphisms of the *RAD51* gene and the g.96267 A>C and the g.85394 A>G polymorphisms of the *BLM* gene and breast cancer occurrence. Genotypes were determined in DNA from peripheral blood by PCR-RLFP and by PCR-CTPP. We observed an association between breast cancer occurrence and the T/G genotype (OR 4.41) of the c.-61 G>T-RAD51 polymorphism, the A/A genotype (OR 1.69) of the g.85394 A>G-BLM polymorphism and the A/A genotype (OR 2.49) of the g.96267 A>C-BLM polymorphism. Moreover, we demonstrated a correlation between intra- and intergenes genotypes combinations and breast cancer occurrence. We found a correlation between progesterone receptor expression and the T/G genotype (OR 0.57) of the c.-61 G>T- *RAD51* polymorphism. We also found a correlation between the T/G genotype (OR 1.86) and the T/T genotype (OR 0.56) of the c.-61 G>T- *RAD51* polymorphism and the lymph node metastasis. We showed an association between the A/A genotype (OR 2.45) and the A/C genotype (OR 0.41) of the g.96267 A>C-BLM polymorphism and G3 grade of tumor. Our results suggest that the variability of the RAD51 and BLM genes may play a role in breast cancer occurrence. This role may be underlined by a common interaction between these genes.

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Introduction

Breast cancer is one of the major causes of cancer death among women in the world. Progress in many fields of science has enabled to understand the mechanism of carcinogenesis in breast, but so far there has not been found any clear reason of breast cancer. Two main groups of breast cancer risk factors are genetic and environmental ones, among which the most important are age and breast cancer occurrence in the first and/or second step relatives. In this case, particularly important are mutations in two high penetrance genes *BRCA1* and *BRCA2* [1, 2], which are associated with the processes of DNA double strand breaks (DSBs) repair. A number of studies show link between hereditary as well as sporadic breast cancer and genetic instability caused by DSBs [3–6]. DSBs can be repaired by nonhomologous end-joining (NHEJ) [7], homologous recombination repair (HRR) [8] and single-strand annealing (SSA) [9]. However, HRR has been found to be a key pathway in human cells for the repair of DSBs [10]. DNA repair by homologous recombination is particularly important during and following DNA replication, when a sister chromatid is present as a template for repair. In eukaryotes, the central homologous recombination protein is RAD51 which catalyses strand transfer between a broken sequence and its undamaged homologue to allow re-synthesis of the damaged region [10–13]. BLM helicase physically and functionally interacts with RAD51 and has been reported to displace RAD51 from the nucleoprotein filament that is responsible for homology searching and strand invasion [14, 15]. BLM activity in relation to RAD51 protein depends on the conformation of the RAD51-ssDNA (single strand DNA) nucleofilaments which are formed by RAD51 protein

in presence of RPA protein and ATP. BLM can destabilize RAD51-ssDNA nucleofilaments if they are in an inactive form, that is, after joining the ADP. When the RAD51-ssDNA nucleofilaments are in an active form, after the ATP joining, BLM stimulates strand exchange carried out by RAD51 [16, 17].

Loss of RAD51 and BLM proteins may contribute to an increased number of somatic mutations and rearrangements of chromosomes. It also contributes to abnormal chromosome segregation, aneuploidy, chromosomal instability, sensitivity to DNA damaging agents and loss of heterozygosity. All the disturbances can lead to reduction of the efficiency of DNA repair processes, cause genomic instability and finally lead to the development of cancer [18]. In breast cancer patients decreasing of the amount of RAD51 protein was observed in 30 % of the cases [19] whereas loss of heterozygosity was observed in 32 % of the cases [20]. The risk of malignant transformation in cells with deficiency of BLM is a hundred times greater than in cells containing a fully functional BLM helicase [21]. On the other hand, different variants of naturally occurring polymorphisms of genes involved in DNA repair can contribute to changes in the efficiency of the repair processes, which may result in the development of cancer [5, 22–27].

The fact that RAD51 and BLM proteins are acting together during DNA repair by homologous recombination prompted us to examine a correlation between polymorphic variants (SNPs) of the *RAD51* gene (c.-61 G>T, rs 1801321 and g.38922 C>G, rs 4417527) and the *BLM* gene (g.96267 A>C, rs 2270132 and g.85394 A>G, rs 2380165) and breast cancer risk. We also studied an association between these polymorphisms of the *RAD51* and *BLM* genes and clinical characteristics of breast cancer patients such as lymph node status, tumor grade, hormone receptors (estrogen and progesterone receptors) and epidermal growth factor receptor (HER2) expression.

Table 1 The clinical characteristics of breast cancer patients

Characteristic	Patients (number/percentage)
Age	
Range: 32–92	304/100
Mean age ± SD: 60±11	
Carcinoma ductale	237/78
Carcinoma intraductale	12/4
Carcinoma lobulare	37/12
Carcinoma medullare	1/0.3
Carcinoma metaplasticum	3/1
Carcinoma metatypicum	1/0.3
Carcinoma mucinosum	9/3
Carcinoma papillare	2/0.7
Carcinoma tubulare	2/0.7
Node	
Positive	89/29
Negative	114/38
Not determined	101/33
Tumor grade by Bloom-Richardson grading system	
1	17/6
2	94/31
3	104/34
Not determined	89/29
ER	
Positive	188/62
Negative	73/24
Not determined	43/14
PR	
Positive	167/55
Negative	94/31
Not determined	43/14
HER2	
Positive	50/16
Negative	187/62
Not determined	67/22

Materials and Methods

Patients

Blood samples were obtained from 304 women (mean age 60 years) with sporadic breast cancer treated at the Department of Surgical Oncology, N. Copernicus Hospital (Lodz, Poland). The clinical characteristic of breast cancer patients is presented in Table 1. Blood was collected before surgical treatment and chemotherapy. The control group (319 women) consisted of age-matched women who were not diagnosed with cancer and recruited from Commune Health Clinic in Rzgow and Institute Polish Mother's Health Center (Lodz, Poland). The Local Ethic Committee approved the study and each patient gave a written consent.

Genomic DNA Isolation

Genomic DNA was prepared from peripheral blood of breast cancer patients and healthy individuals by using of commercial Blood Genomic DNA Miniprep Kit (Axygen Biosciences, CA, USA), as recommended by the manufacturer.

Selection of Polymorphisms and Primers Design

We obtained a list of SNPs in the *RAD51* and *BLM* genes from the public domain of the National Center for Biotechnology Information the Single Nucleotide Polymorphisms database (NCBI dbSNP) at http://www.ncbi.nlm.nih.gov/snp. For this

study we chose the c.-61 G>T (rs1801321) and g.38922G>C (rs4417527) polymorphisms of the *RAD51* gene and the g.96267 A>C (rs2270132) and g.85394 A>G (rs2380165) polymorphisms of the *BLM* gene with a minor allele frequency (MAF) 0.467, 0.109, 0.376 and 0.333 in European population, respectively (submitter population ID: HapMap-CEU for all; http://www.ncbi.nlm.nih.gov/snp). Primers were designed according to the published nucleotide sequence in ENSEMBL database (*RAD51* gene ID: ENSG00000051180 and *BLM* gene ID: ENSG00000197299) and using Primer3 software for c.-61 G>T, g.38922 G>C, g.96267 A>C SNPs (http://frodo.wi.mit.edu/) and Web-based allele-specific primer software for g.85394 SNP (http://bioinfo.biotec.or.th/WASP).

Genotype Determination

The restriction fragment length polymorphism reaction (PCR-RFLP) was used to determine the genotypes of the c.-61 G>T and the g.38922 C>G polymorphisms of the *RAD51* gene and the g.96267 A>C polymorphism of the *BLM* gene. The polymerase chain reaction with confronting two-pair primers (PCR-CTPP) was used to determine the genotypes of the g.85394 A>G polymorphism of the *BLM* gene.

PCR reaction was performed in a total reaction volume of 25 μl containing 50 ng of genomic DNA, 1 U Biotools DNA polymerase (Biotools, Madrid, Spain), 1 × reaction buffer (750 mM Tris-HCl (pH 9.0), 500 mM KCl, 200 mM $(NH_4)_2SO_4$), 0.2 mM of each dNTP, 1.5 mM $MgCl_2$ and 0.25 μM of each primer (Metabion, Martinsried, Germany). The primer sequences are presented in Table 2. PCR amplifications were conducted in DNA Engine thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). Thermal cycling conditions were as follows: initial denaturation step at 95 °C for 5 min, 34 cycles at 95 °C for 30 s, 30 s at 65 °C annealing temperature and 60 s at 72 °C.

The products of the c.-61 G>T and the g.38922 C>G polymorphisms of the *RAD51* gene and the g.96267 A>C polymorphism of the *BLM* gene were digested overnight with 0.2 U of the restriction enzyme *Ngo*MIV, *Nla*III and *Sty*I (NEB New England Biolabs, Ipswich, MA, USA), respectively. The PCR products were separated into 8 % polyacrylamide gel, stained with ethidium bromide and viewed under UV light. Figure 1 presents a representative gels from analysis of the c.-61 G>T (A) and the g.38922 C>G (B) polymorphisms of the *RAD51* gene and the g.96267 A>C (C) and the g.85394 A>G (D) polymorphisms of the *BLM* gene.

Statistical Analysis

Statistical analysis was performed using STATISTICA 8.0 package (Statsoft, Tulsa, OK, USA). Distributions of genotypes and alleles between groups were tested using the χ^2 analysis. The Hardy-Weinberg equilibrium was checked using the χ^2 test to compare the observed genotype frequencies with the expected frequencies among the case and control subjects. For each SNP, the odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated. A linkage between genotype, cancer and clinical parameters was assessed by the logistic regression.

Results

Genotype Analysis

Breast cancer patients and controls were divided into groups corresponding to three genotypes. The genotype distributions

Table 2 Primer sequences of the c.-61 G>T and the g.38922 C>G polymorphisms of the *RAD51* gene and the g.96267 A>C and the g.85394 A>G polymorphisms of the *BLM* gene

Gene	Primer sequences
RAD51 gene	
c.-61 G>T; rs 1801321	
Sense	5′-TGGGAACTGCAACTCATCTGG-3′
Antisense	5′-GCTCCGACTTCACCCCGCCGG-3′
g.38922 C>G; rs 4417527	
Sense	5′-GGCTATTTGGCCAGATTGATAG-3′
Antisense	5′-TCCGGAGTAGCTGGGACTAC-3′
BLM gene	
g.96267 A>C; rs 2270132	
Sense	5′-CAGGCTCCCGGATTCTACTC-3′
Antisense	5′-TGTACACCCCCTAGACG-3′
g.85394 A>G; rs 2380165	
Sense	F1 5′-GGGAGGGCTGCATACACAGAAGC-3′
	F2 5′-GGGGATAGCTGAAGGAAATGAGAAAGGAAACTA-3′
Antisense	R1 5′-GGTGGGAACCTGTGGGGTGC-3′
	R2 5′-TCAACGCTCTTTTCATCATCTCCTGCC-3′

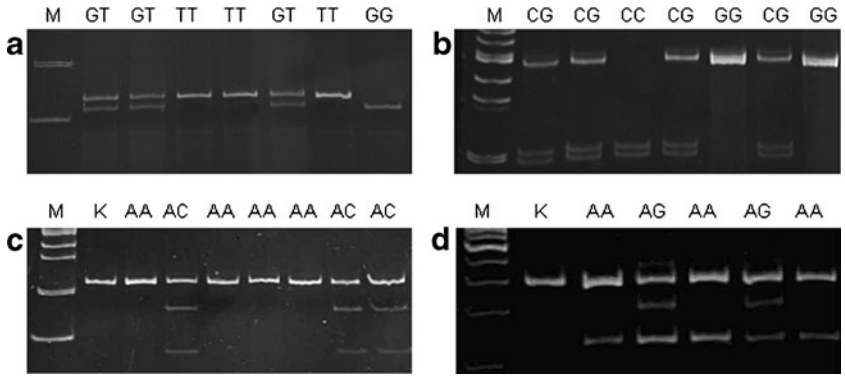

Fig. 1 Genotypes of the c.-61 G>T (rs1801321) (**a**) and the g.38922 C>G (rs4417527) (**b**) polymorphisms of the *RAD51* gene and the g.96267 A>C (rs2270132) (**c**) and the g.85394 A>G (rs2380165) (**d**) polymorphisms of the *BLM* gene analyzed by a 8 % polyacrylamide gel electrophoresis stained with ethidium bromide and viewed under UV light. Lane M shows GeneRuler TM 100 bp molecular weight marker; lane K on the picture C shows a control comprising PCR product without reaction with *Sty*I restriction enzyme; lane K on the picture D shows a control comprising reaction mixture with F1 and R1 starters; all remaining lanes present genotypes indicated in the upper part of the pictures

for the c.-61 G>T and g.38922 C>G polymorphisms of the *RAD51* gene were in agreement with those predicted by the Hardy-Weinberg equilibrium ($p>0.05$), except for that of the c.-61 G>T polymorphism for the patients group. It is caused by very low presence of the G/G genotype of the c.-61 G>T polymorphism of the *RAD51* gene in the Polish population. In the case of genotype distributions for the g.96267 A>C and g.85394 A>G polymorphisms of the *BLM* gene they differed significantly from those predicted by the Hardy-Weinberg equilibrium ($p<0.05$), which can be caused by lack of the G/G genotype of the g.85394 A>G polymorphism and the C/C genotype of the g.96267 A>C polymorphism of the *BLM* gene in the Polish population. The distribution of genotypes of polymorphic variants of the *RAD51* and *BLM* genes and intragene and intergene genotypes combinations for cancer patients and controls is shown in Table 3.

Clinical Parameters of Breast Cancer Patients and BLM and RAD51 Genes Polymorphism

We checked the distribution of genotypes and frequency of alleles of the *BLM* and *RAD51* genes polymorphisms in groups of patients with different hormone receptor status, patients with positive and negative lymph node status and patients with different tumor grade (Table 4). We did not observe any association between estrogen hormone receptor status and the distribution of genotypes and frequency of alleles for four analyzed polymorphisms (data not shown). We did not observe any association between HER2 expression and the distribution of genotypes and frequency of alleles for any polymorphism (data not shown).

We did not observe any correlation between the g.38922 C>G polymorphism of the *RAD51* gene and the g.85394 A>G and the g.96267 A>C polymorphisms of the *BLM* gene and lymph node status (data not shown).

Next of the analyzed clinical features was the tumor grade described by Bloom-Richardson grading system (Table 4). We did not find any association between the c.-61 G>T and the g.38922 C>G polymorphisms of the *RAD51* gene and the g.85394 A>G polymorphism of the *BLM* gene and tumor grade (data not shown).

Discussion

RAD51 recombinase and BLM helicase are the elements of the protein machinery performing DNA DSBs repair by homologous recombination [15]. Homologous recombination is initiated by the MRN complex (MRE11, RAD50, NBN) resecting the DSB ends to generate 3′ overhangs flanking the break site. The resulting ssDNA ends are first protected by RPA before eventually being coated with RAD51 protein to form the critical nucleoprotein filament necessary for accurate repair [11]. BLM helicase was identified in a large DNA damage surveillance complex with BRCA1 and MRN complex [28] and has been shown to associate with many homologous recombination proteins, such as topoisomerase IIIα, BLAP75/RMI1 and RAD51 [29]. Cell and biochemical studies have led to the view that BLM has both pro- and anti-recombinogenic functions. Most notable, BLM is important in stabilizing damaged replication forks and repressing aberrant recombination events, as evidenced by the dramatic increase in levels of sister chromatid exchanges (SCEs) and loss of heterozygosity in *BLM* null cells. In contrast, BLM is also predicted to promote homologous recombination by facilitating exonucleolytic resection of DSBs, by stimulating synthesis-dependent strand annealing, and by promoting non-crossover resolution of Holliday

Table 3 The genotype and allele frequency and odds ratios (OR) of the c.-61 G>T and the g.38922 C>G polymorphisms of the *RAD51* gene and the g.85394 A>G and the g.96267 A>C polymorphisms of the *BLM* gene in breast cancer patients and controls

Genotype or allele	Breast cancer patients (n=304)		Controls (n=319)		OR (95 % Cl)	p
	Number	Frequency	Number	Frequency		
RAD51 c.-61 G>T						
T/T	139	0.46	260	0.82	0.19 (0.13–0.27)↓	<0.001
T/G	152	0.50	59	0.18	4.41 (3.07–6.32)↑	<0.001
G/G	13	0.04	0	0	–	–
T	430	0.71	579	0.91	0.20 (0.14–0.28)↓	<0.001
RAD51 g.38922 C>G						
C/C	217	0.71	250	0.78	0.69 (0.48–0.99)↓	0.045
C/G	83	0.27	65	0.20	1.47 (1.01–2.13)↑	0.043
G/G	4	0.01	4	0.01	1.05 (0.26–4.24)	0.945
C	517	0.85	565	0.89	0.72 (0.52–1.02)	0.061
BLM g.85394 A>G						
A/A	231	0.76	208	0.65	1.69 (1.19–2.40)↑	0.003
A/G	73	0.24	111	0.35	0.59 (0.42–0.84)↓	0.003
G/G	0	0	0	0	–	–
A	535	0.88	527	0.83	1.54 (1.12–2.12)↑	0.003
BLM g.96267 A>C						
A/A	55	0.18	26	0.08	2.49 (1.52–4.09)↑	<0.001
A/C	249	0.82	293	0.92	0.40 (0.25–0.66)↓	<0.001
C/C	0	0	0	0	–	–
A	359	0.59	345	0.54	1.22 (0.97–1.53)↑	<0.001
RAD51 (c.-61 G>T and g.38922 C>G)						
TT/CC	105	0.35	207	0.65	0.29 (0.21–0.40)↓	<0.001
TT/CG	33	0.11	51	0.16	0.64 (0.40–1.02)	0.062
TG/CC	104	0.34	43	0.13	3.34 (2.24–4.98)↑	<0.001
TG/CG	45	0.15	14	0.04	3.79 (2.03–7.05)↑	<0.001
BLM (g.85394 A>G and g.96267 A>C)						
AA/AA	43	0.15	20	0.05	2.46 (1.41–4.29)↑	0.001
AA/AC	188	0.62	188	0.59	1.13 (0.82–1.56)	0.458
AG/AC	61	0.20	105	0.33	0.51 (0.36–0.74) ↓	<0.001
RAD51 (c.-61 G>T) and *BLM* (g.96267 A>C)						
TT/AA	23	0.08	22	0.07	1.11 (0.60–2.03)	0.747
TT/AC	116	0.38	238	0.75	0.21 (0.15–0.30)↓	<0.001
TG/AA	29	0.10	4	0.01	8.31 (2.88–23.92)↑	<0.001
TG/AC	123	0.40	55	0.17	3.26 (2.25–4.72)↑	<0.001
RAD51 (c.-61 G>T) and *BLM* (g.85394 A>G)						
TT/AA	101	0.33	173	0.54	0.42 (0.30–0.58)↓	<0.001
TT/AG	38	0.13	87	0.27	0.38 (0.25–0.58)↓	<0.001
TG/AA	120	0.39	35	0.11	5.29 (3.48–8.05)↑	<0.001
TG/AG	32	0.11	24	0.08	1.45 (0.83–2.52)	0.192
RAD51 (g.38922 C>G) and *BLM* (g.96267 A>C)						
GG/AA	32	0.11	19	0.06	1.86 (1.03–3.35)↑	0.040
GG/AC	185	0.61	231	0.72	0.59 (0.42–0.83)↓	0.002

Table 3 (continued)

Genotype or allele	Breast cancer patients (n=304)		Controls (n=319)		OR (95% Cl)	p
	Number	Frequency	Number	Frequency		
GC/AA	22	0.07	7	0.02	3.48 (1.46–8.26)↑	0.005
GC/AC	61	0.20	58	0.18	1.13 (0.76–1.69)	0.550
RAD51 (g.38922 C>G) and *BLM* (g.85394 A>G)						
GG/AA	163	0.54	160	0.50	1.15 (0.84–1.57)	0.387
GG/AG	54	0.18	90	0.28	0.55 (0.38–0.81)↓	0.002
GC/AA	65	0.21	46	0.14	1.61 (1.07–2.45)↑	0.024
GC/AG	18	0.06	19	0.06	0.99 (0.51–1.93)	0.985

junctions [30]. Recently, it was shown that SUMOylation of BLM can affect its interactions with RAD51. SUMOylation of BLM facilitates repair of damaged replication forks by homologous recombination by modulating recruitment of RAD51 or retention at repair sites [31].

Defects in some DNA repair genes are associated with rare human cancer-prone disorders, like Bloom syndrome resulting from a mutation of the *BLM* gene, ataxia telangiectasia and Werner syndrom. Apart from these rare syndromes, deficient DNA repair is suggested as a predisposing factor in familial breast cancer and in some sporadic breast cancers [3–6]. Advances in the understating of genetic predispositions to breast cancer have also been made by screening naturally occurring polymorphisms in DNA repair genes [5, 22–27]. These studies revealed that subtle defects in DNA repair capacity arising from low-penetrance genes or their combinations are modified by other genetically determined or environmental factors and correlate to breast cancer risk.

In the present study we correlated the genetic constitution of breast cancer patients expressed by polymorphic variants of two important homologous recombination genes, *BLM* and *RAD51*, with clinical parameters of patients, including lymph node status, tumor grade, hormone receptors (estrogen and progesterone receptors) and epidermal growth factor receptor 2 (HER2) expression. The c.-61 G>T-*RAD51* polymorphism is located in the 5′-untranslated region, the regulatory promoter element of the *RAD51* gene. It could affect translation efficiency and mRNA stability, leading to changes in RAD51 protein

Table 4 The genotype, allele frequency and odds ratios (OR) of the c.-61 G>T polymorphism of the *RAD51* gene and the g.96267 A>C polymorphism of the *BLM* gene in subjects with breast cancer with different clinical parameters

Genotype or allele	PR receptor negative (n=94)		PR receptor positive (n=167)		OR (95 % Cl)	p
	Number	Frequency	Number	Frequency		
RAD51 gene (c.-61 G>T)						
T/T	47	0.50	66	0.40	1.53 (0.91–2.55)	0.102
T/G	41	0.44	96	0.57	0.57 (0.34–0.95)↓	0.032
G/G	6	0.06	5	0.03	2.21 (0.66–7.44)	0.201
T	135	0.72	228	0.68	1.25 (0.80–1.96)	0.333
Genotype or allele	Node positive (n=89)		Node negative (n=114)		OR (95 % Cl)	p
	Number	Frequency	Number	Frequency		
RAD51 gene (c.-61 G>T)						
T/T	32	0.36	57	0.50	0.56 (0.32–0.99)↓	0.046
T/G	55	0.62	53	0.46	1.86 (1.06–3.27)↑	0.031
T	119	0.67	167	0.73	0.65 (0.39–1.09)	0.101
Genotype or allele	G3 positive (n=95)		G3 negative (n=104)		OR (95 % Cl)	p
	Number	Frequency	Number	Frequency		
BLM gene (g.96267 A>C)						
A/A	23	0.24	12	0.12	2.45 (1.14–5.25)↑	0.021
A/C	72	0.76	92	0.88	0.41 (0.19–0.88)↓	0.021
A	118	0.62	116	0.56	1.30 (0.87–1.94)	0.200

levels, which, in turn, could influence on the activity of the multiprotein DNA-repair complex that includes BRCA1, BRCA2 and RAD51, and leads to breast cancer susceptibility. The g.38922 C>G-*RAD51* polymorphism is located in the intron 10 in the *RAD51* gene. The intron 10 is located between the exons in the core conserved domain that includes Walker A and B motifs, which have the function of ATP binding and hydrolysis activities. Because of the intron localization the g.38922 C>G polymorphism does not affect amino acid coding and therefore probably does not directly affect protein function but it is likely that may play a role in the proper recognition of non-coding element of RNA splicing and thus it may be indirectly responsible for the correct functioning of RAD51 protein. In turn, it could influence the activity of the multiprotein DNA-repair complex that includes BRCA1, BRCA2, RAD51 and other proteins. On the other hand, the observed associations between breast cancer risk and the g.38922 C>G-*RAD51* polymorphism can be interpreted as affecting the mRNA maturation process and in consequence activity of RAD51 protein.

Beyond genotypes, the expression of genes or proteins might also play a role in breast cancer risk. RAD51 overexpression was associated with a higher risk of locoregional recurrence and death. RAD51 overexpression was also significantly associated with shorter locoregional recurrence-free survival and overall survival [32]. Moreover, it was shown, that RAD51 mRNA levels were inversely associated with PR status and the highest levels were detected in ER-positive/PR-negative tumors. The analysis of microarray expression data from 295 breast cancers indicate that RAD51 increased mRNA expression is associated with higher risk of tumor relapse, distant metastases and worst overall survival [33].

The g.96267 A>C polymorphism is located in the intron 20 and the g.85394 A>G polymorphism is located in the intron 17 of the *BLM* gene. These introns separate exons forming the evolutionarily preserved RecQ-like domain. This domain exhibits a high degree of homology with the RecQ protein of *E. coli*. RecQ-like domain and its constituent sub-domains are responsible for the most important functions of BLM helicase [34]. The polymorphic variant g.96267 A>C is located in the intron 20, which separates the exons encoding the HRDC domain (helicase and RNase D-like C-terminal domain), which in RecQ family-like helicases plays the role in recognizing and binding substrates and protein-protein interactions. In the case of BLM helicase, HRDC domain is also involved in the process of movement and developing of the Holliday structure. Therefore, it is likely that the polymorphic variants of the *BLM* gene g.96267 A>C and g.85394 A>G may play an important role in the proper recognition and cutting out introns in the process of mRNA maturation and may be responsible for the correct functioning of BLM helicase.

Our results on the genes polymorphism and breast cancer risk are partly in agreement with the study presented by Ding et al. [26]. Comparing the results of Ding et al. carried out on population of women living in Taiwan to our results we simillarly observed a protective role of the A/C variant in the case of the g.96267 A>C polymorphism of the *BLM* gene (Table 3). On the other hand, our results disagree with those of Ding et al. in the case of the genotype the C/C of the g.38922 C>G polymorphism of the *RAD51* gene (Table 3).

It was shown that the polymorphic variant of the *BLM* gene − rs2380165 and the variants of the *RAD51* gene – rs4417527 and rs2412546 were associated with breast cancer risk [26]. Moreover, not only the intronic polymorphisms located within the region encoding the helicase domain of BLM but also these within the RAD51-interaction domain-encoding region showed an interaction with *RAD51* polymorphisms in determining breast cancer susceptibility. Recently, it was also shown that the domains responsible for RAD51 partnership coincide with the domains responsible for the binding of ssDNA, $BLM_{100–214}$ and $BLM_{1317–1367}$ [29]. This suggests that the ability of BLM to suppress homologous recombination may involve both the displacement of RAD51 from the D-loop and the binding of BLM to the newly liberated ssDNA to reinstate the duplex and completely impede genetic exchange through homologous recombination.

In the present study we also correlated the genotype combinations of four polymorphic variants of the *BLM* and *RAD51* genes with breast cancer occurrence (Table 3). Especially, in the case of analysis of genotype intergene combinations and breast cancer occurrence, we detected a high increase of cancer risk (Table 3).

Relationship between the c.-61 G>T and g.38922 C>G polymorphisms of the *RAD51* gene, and the g.91266 C>A and g.85394 A>G polymorphisms of the *BLM* gene and clinical characteristics of breast cancer patients was also studied (Table 4). Breast cancer patients were divided into groups depending on lymph node status, tumor grade and expression of estrogen and progesterone receptors (ER and PR) and HER2 receptor status. Auxiliary lymph node status and tumor size are clinical parameters directly correlated with the survival period in breast cancer. Expression level of ER and PR are useful predictive markers allowing the assessment of the response to endocrine therapy – ER- and PR-positive tumors may have a 6- to 7-higher response rate to negatives [35].

Next of the analyzed clinical features was the presence of HER2 (C-ERB/neu), belonging to the group of oncogenes of the tyrosine kinase function, which is overexpressed in about 20–30 % of breast cancers and often is associated with the resistance to a hormone therapy. Activation of this receptor leads to the uncontrolled division of cancer cells, which may be accompanied by an increased amount of vascular endothelial growth factor (VEGF) as the main factor inducing the formation of new blood vessels within the tumor. HER2 overexpression leads to early metastases in the direction of the lymph nodes, early recurrences of the

disease and is associated with shorter survival and worse clinical prognosis [36]. We did not observe any association between HER2 expression and the distribution of genotypes and frequency of alleles within any of the analyzed polymorphisms (data not shown).

We also correlated the genotypes of the *BLM* and *RAD51* polymorphic variants with tumor grade described by the Bloom-Richardson grading system (Table 4). It is a three-step scale, according to which the tumor samples taken during biopsy are classified, and then analyzed using a microscope. To the G1 category belong tumors whose cells are small, have regular shapes, show high similarity in structural terms to normal breast cells and their mitotic index does not exceed the number of seventh. To the G2 category belong tumors whose cells exhibit visible changes compared to normal breast cells and their mitotic index ranges from 8 to 14. To the last G3 category belong tumors whose cells are large and have irregular shapes. They differ significantly from normal cells, divide rapidly − their mitotic index exceeds the number 15.

The results obtained indicate that the *RAD51* and *BLM* genes polymorphism can be associated with breast cancer risk. An association may be underlined by a common interaction between these genes. We showed that the c.-61 G>T polymorphism of the *RAD51* gene might modulate breast cancer risk and be associated with progesterone receptor expression and local metastasis. We also found an association between the g.96267 A>C-*BLM* polymorphism and G3 grade of tumor.

Conclusion

The variability of the *RAD51* and *BLM* genes may play a role in breast cancer occurrence. The examined polymorphisms of the *RAD51* and *BLM* genes cannot be an independent markers of breast cancer but our studies may be useful in building a set of clinical and molecular markers helpful for diagnosis of breast cancer.

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