The association between polymorphisms of the RAD51-G135C, XRCC2-Arg188His and XRCC3-Thr241Met genes and clinico-pathologic features in breast cancer in Poland

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Summary

Background: XRCC2 and XRCC3 genes are structurally and functionally related to RAD51 which plays an important role in homologous recombination, the process frequently involved in cancer transformation. *Material and Methods:* In the present work the distribution of genotypes and frequency of alleles of the RAD51 G135C polymorphism, XRCC2 Arg188His and XRCC3 Thr241Met polymorphism in 790 cases of breast cancer were investigated. The control group consisted of 798 cancer-free blood donors (age ± 5 years) who were sex and ethnicity-matched. The polymorphisms were determined by PCR-RFLP methods. We also correlated genotypes with the clinical characteristics of breast cancer patients. *Results:* Our results obtained for the 135G>C polymorphism of the RAD51 gene indicated that both the C/C genotype and the C allele are strongly associated with breast cancer. The Arg/His genotype of XRCC2 (OR = 2.16, 95% CI = 1.48-3.16) and Thr/Met of XRCC3 increased the risk of type I breast cancer occurrence (OR = 2.33,95% CI = 1.60-3.41). We did not find any association with the *RAD51, XRCC2/3* gene polymorphism and estrogen and progesterone receptor status. *Conclusion:* The results support the hypothesis that the polymorphism of RAD51 and XRCC2/3 gene may be associated with the incidence of sporadic breast cancer in Polish women.

Key words: RAD51; XRCC2; XRCC3; Breast cancer; Gene polymorphism.

Introduction

Breast cancer is one of the major killers worldwide. In 2008, breast cancer caused 458,503 deaths worldwide (13.7% of cancer deaths in women). The risk of breast cancer is increased by several factors such as sex, age, lack of childbearing or breastfeeding, higher hormone levels, race, economic status and gene mutations [1, 2].

There is growing evidence that human cancer can be induced by DNA double strand breaks (DSBs) [3].

A double strand break (DSB) is the most lethal of all DNA lesions. If unrepaired, a DSB leads to loss of chromosome segments and threatens the survival of the cell [4, 5].

DSB in DNA are repaired by two major mechanisms: homologous recombination (HR) and nonhomologous end joining (NHEJ) [6, 7].

The RAD51, XRCC2 and XRCC3 proteins are core components of DNA double strand breaks (DSBs) repair by HRR. *XRCC2* and *XRCC3* genes are structurally and functionally related to the *RAD51* gene [8, 9].

A large number of molecular epidemiologic studies have been performed on various neoplasms, such as cancer of the breast, lung, ovarian, bladder, head and neck and skin to evaluate the role of *XRCC2*, *XRCC3* and *RAD51* polymorphisms [10-16].

The G135C, polymorphism in the 5 UTR of the *RAD51* gene has been reported to be associated with altered gene transcription [17].

Two researchers showed previously that this polymorphism was not an independent marker in breast cancer, but it could be associated with an increased gastric cancer risk and an increased breast cancer risk in BRCA2 mutation carriers [18, 19]. Similar results came from other laboratories [20, 21].

A relatively rare polymorphism in the *XRCC2* gene, a G>A transition resulting on Arg to His substitution at codon 188 was found to be related with breast cancer, but not with bladder cancer, colorectal adenoma, skin cancer and endometrial carcinoma [10, 22-26].

Recently, many studies have shown that the *XRCC3* Thr241Met genotype has been linked to an increased risk of breast, colorectal, bladder, and head and neck cancer [27-30].

The literature data suggest that the identification of new risk factors for breast cancer in the population of women is urgently needed, and an analysis of some gene polymorphisms could be an interesting option.

Therefore in the present work the association between polymorphisms in three genes involved in the homologous recombination of double-strand breaks: *RAD51* 5' untranslated region G135C, X-ray repair cross-complementing group 2 (*XRCC2*) Arg188His, and *XRCC3* Thr241Met and breast cancer risk in polish women was investigated.

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Materials and Methods

Clinicopathologic data and genotyping

Blood was obtained from 790 women of Caucasian ethnicity treated at the Department of Oncology, Institute of Polish Mother's Memorial Hospital, Lodz, Poland (from 2000 to 2010). The mean age of the 790 patients at the time of diagnosis was 59 years (range 31-76). The controls were somewhat younger: mean age 54 years (range 44-86). No significant difference was observed in age distribution between the breast cancer patients and healthy controls. The gender distribution in breast cancer patients was also similar to that in the healthy controls.

The diagnosis of cancer was made after histopathological examination of patients' biopsies. Patients with breast cancer involved in the study were analyzed according to TNM Classification of Malignant Tumors which describes the extent of cancer in a patient's body: T describes the size of the tumor and whether it has invaded nearby tissue, N describes regional lymph nodes that are involved and M describes distant metastasis (spread of cancer from one body part to another). There were 320 women with node-negative and 380 with node-positive ductal breast carcinoma. No distant metastases were found in patients at the time of treatment. Median follow-up of patients still at the time of analysis was 39 months (range: 2-71 months). The average tumor size was 20 mm (range 17-32 mm). All tumors were graded by a method based on the criteria of Scarf-Bloom-Richardson. The demographic and pathologic features of patients are summarized in Table 1. All subjects involved in the study were unrelated Caucasians and resided in the Lodz district, Poland. The study was approved by the Local Ethics Committee and written consent from each patient or healthy blood donor was obtained before participating in the study.

Determination of RAD51 genotype

RAD51 genotyping was analyzed by PCR amplification of a 175-bp region around nucleotide 135. This region contained a single *Mva*I site that was abolished in the 135C allele. Wild type alleles were digested by *Mva*I resulting in 86- and 71-bp product. The 135C allele was not digested by the enzyme, resulting in a single 157-bp product. The *RAD51* genotype was analyzed using the specific primers forward 5' TGG GAA CTG CAA CTC ATC TGG 3' and reverse 5' GCG CTC CTC TCT CCA GCAG 3'.

PCR was carried out in a GeneAmp PCR system 9700 (Applied Biosystems) thermal cycler. PCR amplification was performed in a final volume of 25 l. The reaction mixture contained 5 ng genomic DNA, 0.2 mol of each appropriate primer (ARK Scientific GmbH Biosystems, Darmstad, Germany), 2.5 mM $MgCl_2$, 1 mM dNTPs and 1 unit of Taq polymerase (Qiagen GmbH, Hilden, Germany). The PCR cycle conditions were 94°C for 60 sec, 54°C for 30 sec then 72°C for 40 sec, repeated for 35 cycles. After digestion with MvaI for 4h at 37°C samples were run on 7% polyacrylamide gel and visualized by ethidium bromide staining. Each subject was classified into one of the three possible genotypes: G/G, G/C or C/C.

Determination of XRCC2 genotype

Polymorphism of the XRCC2 gene was determined by PCR-RFLP, using primers: forward 5'TGTAGTCACC-CATCTCTCTGC3' and reverse: 5'AGTTGCTGCCATGCCT-TACA3'. The 25 µl PCR mixture contained about 100 ng of DNA, 12.5 pmol of each primer, 0.2 mmol/l of dNTPs, 2 mmol/l of $MgCl_2$ and 1 U of Taq DNA polymerase. The 290 bp amplified product was digested overnight with 1 U of *Hpn*I at 37ºC. The wild-type allele Arg was identified by the presence of two 290 bp bands, while the mutant allele His was represented by 148, and 142 bp bands.

Table 1. — *Pathologic features of patients with breast cancer.*

Breast cancer	Patients (n = 790)	
	n	%
Ductal carcinoma	790	100
Scarf-Bloom-Richardson stage		
I	230	29
II	480	64
III	80	7
Tumor size grade		
T1	110	11
T2	430	57
T3	180	26
T4	70	6
Lymph node status		
N0	350	46
N1	150	17
N2	170	20
N3	70	10
N4	50	7
ER		
Positive	450	57
Negative	340	43
PR		
Positive	450	57
Negative	340	43
HER-2		
Positive	470	41
Negative	320	59

Table 2. — *Distribution of G/G, G/C and C/C genotypes and frequencies of the G and C alleles of RAD51 G135C polymorphism in patients with breast cancer (n = 790) and controls (n = 798).*

	Breast cancer patients		Controls			
	Number	(%)	Number	(%)	OR (95% CI)[a]	p^b
G/G	160	20	208	26	1.00 Ref.	
G/C	104	13	426	53	**0.31 (0.23- 0.43)**	**< .0001**
C/C	526	67	164	21	**4.16 (3.18- 5.46)**	**< .0001**
G	424	27	842	53	1.00 Ref.	
C	1156	73	754	47	**3.04 (2.62- 3.53)**	**< .0001**

Data in boldface are statistically significant.
[a]Crude odds ratio (OR); 95% CI = confidence interval at 95%; [b]Chi square.

Table 3. — *Distribution of Arg/Arg, Arg/His and His/His genotypes and frequencies of the Arg and His alleles of XRCC2 Arg188His polymorphism in patients with breast cancer (n = 790) and controls (n = 798).*

	Breast cancer patients		Controls			
	Number	(%)	Number	(%)	OR (95% CI)[c]	p^b
Arg/Arg	212	27	202	25	1.00 Ref.	
Arg/His	374	47	406	51	0.87 (0.69-1.11)	0.312
His/His	204	26	190	24	1.02 (0.77-1.34)	0.920
Arg	798	51	810	51	1.00 Ref.	
His	782	49	786	49	1.00 (0.88-1.16)	0.920

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

Determination of XRCC3 genotype

Polymorphism of the XRCC3 gene was determined by PCR-RFLP, using codon 241 primers (5'-GCCTGGTGGTCATC-

Table 4. — *Distribution of Thr/Thr, Thr/Met and Met/Met genotypes and frequencies of the Thr and Met alleles of XRCC3 Thr241Met polymorphism in patients with breast cancer (n = 790) and controls (n = 798).*

	Breast cancer patients		Controls			
	Number	(%)	Number	(%)	OR (95% CI)[c]	p[b]
Thr/Thr	220	28	188	24	1.00 Ref.	
Thr/Met	378	48	384	48	0.84 (0.66-1.07)	0.178
Met/Met	192	24	226	28	**0.72 (0.55-0.95)**	**0.025**
Thr	818	52	760	48	1.00 Ref.	
Met	762	48	836	52	**0.84 (0.73-0.97)**	**0.021**

Data in boldface are statistically significant.
[a]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [b]Chi square.

GACTC-3' and 5'-ACAGGGCTCTGGAAGGCACT GCTCAGCTCACGCACC- 3'). The 25 µl PCR mixture contained about 100 ng of DNA, 12.5 pmol of each primer, 0.2 mmol/L of dNTPs, 2 mmol/L of $MgCl_2$ and 1 U of Taq DNA polymerase. The 552 bp amplified product was digested overnight with 5 U of *Nla*III at 37ºC. The wild-type allele Thr was identified by the presence of two 239 and 313 bp bands, while the mutant allele Met was represented by 105, 208, and 239 bp bands.

Immunohistochemistry

Tumor tissue biopsies were fixed in formalin and embedded in paraffin according to standard procedures. Immunohistochemistry staining was performed as described by Sannino and Shousha [31] The primary antibodies against ER (clone 1D5), PR (clone 1A6) and HER-2 (polyclonal anti-human HER-2) were purchased from DAKO Corporation (CA, USA).

Statistical analysis

For each polymorphism, deviation of the genotype frequencies in the controls from those expected under Hardy-Weinberg equilibrium was assessed using the standard χ^2-test. Genotype frequencies in cases and controls were compared by c2-tests. The genotypic-specific risks were estimated as odds ratios (ORs) with associated 95% intervals (CIs) by unconditional logistic regression. *P* values < 0.05 were considered to be significant. STATISTICA 6.0 software (Statsoft, Tulsa, OK, USA) was used to perform analyses.

Results

All the recruited samples were successfully genotyped for the *RAD51* polymorphism. Genotype and allele frequencies for cases and controls are presented in Table 2. There were significant differences ($p < 0.05$) between the two investigated groups. The women with breast cancer showed an incidence of 20, 13, and 67%, respectively, for the G/G, G/C, and C/C genotypes of the *RAD51* gene, whereas the control group showed 26, 53, and 21% for the same genotypes. In patients the observed frequencies of the G/G, G/C and C/C genotypes differed significantly ($p < 0.05$) from the distribution expected from the Hardy-Weinberg equilibrium The C/C genotype frequency was statistically significant with an OR of 4.16 and 95% CI of (3.18-5.46) (Table 2). Variant 135C allele of *RAD51* increased cancer risk [OR = 3.04 (2.62-3.53) $p < .0001$].

Table 5. — *Dependency of genotypes and frequencies of the alleles of RAD51, XRCC2 and XRCC3 gene polymorphism on the tumour stage in patients with breast cancer*[a].

Stage[b]	I (n = 230)	II+III (n = 560)	OR (95% CI)[c]	p[d]
RAD51 G135C	Number (%)	Number (%)		
G/G	50 (22%)	110 (20%)	1.00 Ref.	
G/C	26 (11%)	78 (14%)	0.73 (0.42-1.27)	0.337
C/C	154 (67%)	372 (66%)	0.91 (0.62-1.34)	0.708
G	126 (27%)	298 (27%)	1.00 Ref.	
C	334 (73%)	822 (73%)	0.96 (0.75-1.22)	0.791
XRCC2 Arg188His				
Arg/Arg	50 (22%)	162 (29%)	1.00 Ref.	
Arg/His	150 (65%)	224 (40%)	**2.16 (1.48-3.16)**	**< .0001**
His/His	30 (13%)	174 (31%)	**0.55 (0.33-0.92)**	**0.029**
Arg	250 (54%)	548 (49%)	1.00 Ref.	
His	210 (46%)	572 (51%)	0.80 (0.64-1.00)	0.057
XRCC3 Thr241Met				
Thr/Thr	50 (22%)	170 (30%)	1.00 Ref.	
Thr/Met	154 (67%)	224 (40%)	**2.33 (1.60-3.41)**	**< .0001**
Met/Met	26 (11%)	166 (30%)	**0.53 (0.31-0.89)**	**0.023**
Thr	254 (55%)	564 (50%)	1.00 Ref.	
Met	206 (45%)	556 (50%)	0.82 (0.66-1.02)	0.089

Data in boldface are statistically significant.
[a]n = 790; [b]according to Scarf-Bloom-Richardson criteria; [c]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [d]Chi square.

No statistically significant differences were observed in the alleles or in the genotype frequencies of the *XRCC2* Arg188His gene polymorphisms between the control group and breast cancer patients (Table 3).

However, a weak association between breast cancer occurrence and Met/Met genotype (OR = 0.72 (0.55-0.95) and the Met allele (OR = 0.84 (0.73-0.97) of *XRCC3* Thr241Met was observed. The increase was statistically significant ($p < 0.05$ for both) (Table 4).

When stratified for histology types and clinical stage breast cancer, frequencies of the *RAD51*, *XRCC2* and *XRCC3* genotypes among patients were also significantly different from controls.

Clinicopathological parameters

The histological stage was evaluated in all cases (n = 790). Stage II and III were grouped together for the purposes of statistical analysis (Table 5). There was a correlation between genotypes of the two polymorphisms *XRCC2*-Arg188His and *XRCC3*-Thr241Met and breast cancer invasiveness. We found statistically significant increase of His/His [OR = 0.55 (0.33-0.92), $p = 0.029$] and Met/Met homozygotes frequency [OR = 0.53 (0.31-0.89), $p = 0.023$] in the Stage I group by the Scarf-Bloom-Richardson classification. The higher risk of breast carcinoma of Stage I occurrence was associated with the Arg/His [OR = 2.16 (1.48-3.16), $p < 0.0001$] and Thr/Met genotype [OR = 2.33 (1.60-3.41), $p < 0.0001$].

The distribution of genotypes and frequency of alleles in patients with lymph node metastasis (N+) and without (N–) is displayed in Table 6. We did not observe any differences in the distribution of genotypes of investigated polymorphisms between these groups. Additionally, there was no difference in distribution of genotypes and frequency of alleles in group of patients with different tumor sizes.

Table 6. — *RAD51, XRCC2 and XRCC3 gene polymorphism and breast cancer progression*[a].

	Tumor size			Node status		
	T3+T4 N = 250	T1+T2 N = 540	OR (95% CI)[a]	N+ (n = 440)	N– (n = 350)	OR (95% CI)[b]
RAD51 G135C	Number (%)	Number (%)		Number (%)	Number (%)	
G/G	57 (22%)	103 (20%)	1.00 Ref.	87 (20%)	73 (22%)	1.00 Ref.
G/C	33 (11%)	71 (12%)	0.83 (0.49-1.41)	51 (12%)	53 (11%)	0.80 (0.49-1.32)
C/C	160 (67%)	366 (68%)	0.79 (0.54-1.14)	302 (68%)	224 (67%)	1.13 (0.79-1.61)
G	147 (29%)	277 (26%)	1.00 Ref.	225 (26%)	199 (28%)	1.00 Ref.
C	353 (71%)	803 (74%)	0.82 (0.65-1.04)	655 (74%)	501 (72%)	1.15 (0.92-1.44)
XRCC2 Arg188His						
Arg/Arg	64 (37%)	148 (26%)	1.00 Ref.	120 (37%)	92 (15%)	1.00 Ref.
Arg/His	122 (40%)	252 (50%)	1.11 (0.77-1.61)	198 (40%)	176 (55%)	0.86 (0.61-1.21)
His/His	64 (22%)	140 (24%)	1.05 (0.69-1.06)	122 (22%)	82 (30%)	1.14 (0.77-1.68)
Arg	250 (50%)	548 (51%)	1.00 Ref.	438 (50%)	360 (51%)	1.00 Ref.
His	250 (50%)	532 (49%)	1.03 (0.83-1.27)	442 (50%)	340 (49%)	1.06 (0.87-1.30)
XRCC3 Thr241Met						
Thr/Thr	77 (34%)	143 (25%)	1.00 Ref.	126 (29%)	94 (29%)	1.00 Ref.
Thr/Met	124 (42%)	254 (51%)	0.90 (0.63-1.28)	194 (44%)	184 (44%)	0.78 (0.56-1.09)
Met/Met	49 (24%)	143 (24%)	0.63 (0.41-0.97)	120 (27%)	72 (27%)	1.24 (0.83-1.74)
Thr	278 (56%)	540 (50%)	1.00 Ref.	446 (51%)	372 (53%)	1.00 Ref.
Met	222 (44%)	540 (50%)	0.79 (0.64-0.98)	434 (49%)	328 (47%)	1.10 (0.90-1.34)

[a]T2 vs T3+T4, [b]N- (node negative) vs N+ (node positive).

Hormone receptor status

Distributions of genotypes of the three polymorphisms of DNA repair gene for cancer patients with different hormone receptor status is shown in Table 7. We did not find any association with the *RAD51*, *XRCC2/3* gene polymorphism and estrogen and progesterone receptor status for breast cancer patients.

Discussion

The present study examined whether polymorphism Arg188His of *XRCC2*, Thr241Met of *XRCC3* and 135G/C of *RAD51* gene is related to the development of breast cancer. We found an association between breast cancer and three investigated polymorphisms in this study population.

The single nucleotide polymorphism (SNP) has been studied as a risk factor for various cancers [10-16].

The polymorphisms chosen for this study have been shown to have functional significance and may be responsible for a low DNA repair capacity phenotype characteristic of cancer patients including breast carcinoma.

A single nucleotide polymorphism, 135G/C, is located in the 5'-untranslated region of the *RAD51* gene, so it could affect the gene expression and, as a consequence, alter the concentration of the final product-the RAD51 protein. RAD51 takes part in the repair of DNA double strand breaks (DSBs), the most dangerous damage to DNA. The formation of RAD51 foci represents an important step in the repair of DNA double-strand breaks.

Recently, several studies have shown that the 135G>C polymorphism can modify the effect of polymorphisms of the *BRCA2* and *XRCC3* genes on breast cancer occurrence [32-35].

In the literature the 135G/C polymorphism of *RAD51* gene may be identified as a susceptibility locus for breast cancer [36, 37].

CC genotype may be associated with an elevated tumor risk in European populations in sporadic breast cancer [36, 38].

Our results is in line with data of other reports introducing the important role of *RAD51* G135C polymorphism for breast carcinoma occurrence.

In our work CC genotype was associated with an increased risk for the development of breast cancer compared with the GG and GC genotype. The C allele also increased the risk of breast cancer compared with the G allele.

In our earlier study *RAD51* G135C polymorphism was not related to breast cancer occurrence. The reason for this could be the relatively small group of patients enrolled in our study [39].

We also analyzed the distribution of genotypes and frequency of alleles in groups of patients suffering from breast cancer according to different cancer staging by the Scarf-Bloom-Richardson classification. The heterozygote Thr241Met genotype was associated with type I breast cancer. The similar relationships for Arg188His genotype of *XRCC2* were statistically significant.

We found however in the literature, other data. No significant associations were observed between the Thr241Met and breast cancer in Cypriot women [40].

In the Polish population the Thr241Met genotype of the *XRCC3* polymorphism slightly increased the risk of local metastasis in breast cancer patients [41, 42]. Moreover, the combined Thr241Met-XRCC3/135G/C-RAD51 genotype decreased the risk of breast cancer occurrence [41].

Similarly to our observation, according to recent reports, the *XRCC3* Thr241Met allele seems to be associated with elevated breast cancer risk in non-Chinese subjects [43].

The role of position 188 in the functioning of the XRCC2 protein is unknown. Indeed, very few sites of functional significance within the protein have been iden-

Table 7. — . *Genotype distribution and odds ratios (OR) of the RAD51, XRCC2 and XRCC3 polymorphism gene for breast cancer patients with different hormone receptor status.*

Stage[b]	ER– (n = 340)	ER+ (n = 450)	OR (95% CI)[c]	*p*[d]
RAD51 G135C	Number (%)	Number (%)		
G/G	70 (13%)	90 (20%)	1.00 Ref.	
G/C	50 (5%)	54 (12%)	1.19 (0.72-1.95)	0.571
C/C	220 (82%)	306 (68%)	0.92 (0.64-1.32)	0.729
G	190 (28%)	234 (26%)	1.00 Ref.	
C	490 (72%)	666 (74%)	0.90 (0.72-1.13)	0.420
XRCC2 Arg188His				
Arg/Arg	94 (28%)	118 (26%)	1.00 Ref.	
Arg/His	152 (44%)	222 (50%)	0.85 (0.61-1.20)	0.431
His/His	94 (28%)	110 (24%)	1.07 (0.72-1.57)	0.791
Arg	250 (50%)	458(51%)	1.00 Ref.	
His	250 (50%)	442 (49%)	1.03 (0.83-1.28)	0.791
XRCC3 Thr241Met				
Thr/Thr	99 (29%)	121 (27%)	1.00 Ref.	
Thr/Met	151 (44%)	227 (50%)	0.81 (0.58-1.13)	0.261
Met/Met	90 (27%)	102 (23%)	1.07 (0.73-1.59)	0.777
Thr	349 (51%)	469 (52%)	1.00 Ref.	
Met	331 (49%)	431 (48%)	1.09 (0.89-1.33)	0.416
	PR– (n = 310)	PR+ (n = 480)	OR (95% CI)[c]	*p*[d]
RAD51 G135C	Number (%)	Number (%)		
G/G	62 (20%)	98 (20%)	1.00 Ref.	
G/C	39 (13%)	65 (14%)	0.94 (0.57-1.57)	0.920
C/C	209 (67%)	317 (66%)	1.04 (0.72-1.49)	0.887
G	163 (26%)	261(27%)	1.00 Ref.	
C	457 (74%)	699 (73%)	1.04 (0.83-1.31)	0.740
XRCC2 Arg188His				
Arg/Arg	84 (27%)	128 (27%)	1.00 Ref.	
Arg/His	142 (46%)	232 (48%)	0.93 (0.66-1.31)	0.764
His/His	84 (27%)	120 (25%)	1.06 (0.72-1.57)	0.823
Arg	310 (50%)	488 (51%)	1.00 Ref.	
His	310 (50%)	472 (49%)	1.03 (0.84-1.26)	0.791
XRCC3 Thr241Met				
Thr/Thr	89 (29%)	131 (28%)	1.00 Ref.	
Thr/Met	141 (45%)	237 (49%)	0.87 (0.62-1.23)	0.497
Met/Met	80 (26%)	112 (23%)	1.05 (0.70-1.55)	0.887
Thr	319 (51%)	499 (52%)	1.00 Ref.	
Met	301 (49%)	461 (48%)	1.02 (0.83-1.25)	0.887
	HER– (n = 320)	HER+ (n = 470)	OR (95% CI)[c]	*p*[d]
RAD51 G135C	Number (%)	Number (%)		
G/G	64 (20%)	96 (20%)	1.00 Ref.	
G/C	44 (14%)	60 (13%)	1.1 (0.66-1.81)	0.806
C/C	212 (66%)	314 (67%)	1.01 (0.70-1.45)	1.000
G	172 (27%)	252 (27%)	1.00 Ref.	
C	468 (73%)	688 (73%)	0.99 (0.79-1.25)	1.000
XRCC2 Arg188His				
Arg/Arg	87 (27%)	125 (27%)	1.00 Ref.	
Arg/His	145 (45%)	229 (49%)	0.90 (0.64-1.28)	0.654
His/His	88 (28%)	116 (25%)	1.09 (0.73-1.60)	0.740
Arg	319 (50%)	479 (51%)	1.00 Ref.	
His	321 (50%)	461 (49%)	1.04 (0.85-1.27)	0.698
XRCC3 Thr241Met				
Thr/Thr	93 (29%)	127 (27%)	1.00 Ref.	
Thr/Met	143 (44%)	235 (50%)	0.83 (0.59-1.16)	0.324
Met/Met	84 (27%)	108 (23%)	1.06 (0.71-1.57)	0.841
Thr	329 (51%)	489 (52%)	1.00 Ref.	
Met	311 (49%)	451 (48%)	1.02 (0.83-1.25)	0.841
	PR+ or ER+ or HER+	PR– and ER– and HER–	OR (95% CI)[c]	*p*[d]
RAD51 G135C	Number (%)	Number (%)		
G/G	70 (20%)	96 (20%)	1.00 Ref.	
G/C	61 (17%)	65 (14%)	1.28 (0.80-2.05)	0.345
C/C	220 (63%)	314 (66%)	0.96 (0.67-1.36)	0.887
G	201 (29%)	257 (27%)	1.00 Ref.	
C	501 (71%)	693 (73%)	0.92 (0.74-1.14)	0.511
XRCC2 Arg188His				
Arg/Arg	84 (27%)	125 (27%)	1.00 Ref.	
Arg/His	142 (46%)	222 (49%)	0.95 (0.67-1.34)	0.841
His/His	84 (27%)	110 (24%)	1.13 (0.76-1.68)	0.596
Arg	310 (50%)	472 (52%)	1.00 Ref.	
His	310 (50%)	442 (48%)	1.11 (0.91-1.37)	0.307
XRCC3 Thr241Met				
Thr/Thr	121 (26%)	89 (29%)	1.00 Ref.	
Thr/Met	235 (52%)	141 (45%)	1.22 (0.86-1.72)	0.283
Met/Met	102 (22%)	80 (26%)	0.93 (0.62-1.04)	0.841
Thr	477 (52%)	319 (51%)	1.00 Ref.	
Met	439 (48%)	301 (49%)	0.97 (0.79-1.19)	0.841

[a]n = 790; baccording to Scarf-Bloom-Richardson criteria; [c]Crude odds ratio (OR), 95% CI.

tified [44, 45]. Rafii *et al*. suggested that *XRCC2* Arg188His polymorphism is not directly associated with breast cancer risk [44].

In conclusion our study is one of individual reports on the significance of Thr241Met and Arg188His genotype with tumor staging in carcinoma of the breast.

However we did not find any association between clinicopathological characteristics of breast cancer patients: lymph node status, hormone receptors (estrogen and progesterone receptors) and epidermal growth factor receptor (HER2) expression and polymorphism of the *RAD51* and *XRCC2/3* gene.

Finally we suggested that Arg188His, Thr241Met and the G135C polymorphisms may be associated with the occurrence of breast cancer in Poland. Further studies, conducted on a larger group, are required to clarify this point.

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