Single nucleotide polymorphism (SNP) Thr241Met in the *XRCC3* gene and breast cancer risk in Polish women

Hanna Romanowicz-Makowska[1], Magdalena Bryś[2], Ewa Forma[2], Rafał Maciejczyk[3], Ireneusz Połać[4], Dariusz Samulak[5], Magdalena Michalska[5], Beata Smolarz[1]

[1]Laboratory of Molecular Genetics, Department of Pathology, Institute of Polish Mother's Memorial Hospital, Lodz, Poland
[2]Department of Cytobiochemistry, University of Lodz, Poland
[3]Department of Oncology, Institute of Polish Mother's Memorial Hospital, Lodz, Poland
[4]Department of Menopausal Diseases, Institute of Polish Mother's Memorial Hospital, Lodz, Poland
[5]Clinic of Gynaecological Surgery, Poznan University of Medical Sciences, Poland

Background: Single nucleotide polymorphisms (SNPs) in DNA repair genes may be associated with differences in the repair efficiency of DNA damage and may influence an individual's risk of cancer. The XRCC3 protein plays a critical role in Homologous Recombination Repair (HRR) accounting for repair of DNA double-strand breaks (DSB).
Aim: The aim of the present study was to evaluate associations between the risk of breast cancer and Thr241Met polymorphism in the *XRCC3* gene.
Material and methods: Single nucleotide polymorphism was genotyped by the PCR-RFLP (restriction fragment-length polymorphism) method in 760 women with sporadic breast cancer and in 760 control samples. The present study confirmed a relationship between *XRCC3* Thr241Met polymorphism and breast cancer progression, assessed by the degree of lymph node metastases and histological stages.
Conclusion: Our findings suggest that the analysis of *XRCC3* polymorphism, may contribute to better understanding of the mechanisms of breast cancer by evaluating possible interactions between these genotypes and well-established risk factors for breast cancer.

Key words: XRCC3, breast cancer, gene polymorphism.

Introduction

Breast cancer is the most frequent cancer in women worldwide. The highest incidence rates are observed in North America, whereas the lowest risk of breast cancer is observed in Asia and Africa [1]. Breast cancer is also the most common cancer in females in Europe. The regions of highest incidence are Western and Northern Europe, while Southern and Eastern Europe have lower incidence rates.

It has been suggested that sporadic breast cancer is most likely caused by low-penetrance genes, including those involved in DNA repair mechanisms. Furthermore, the accumulation of DNA damage may contribute to breast carcinogenesis.

Different DNA repair pathways play a vital role in preserving genome stability and genetic variations in multiple repair pathways may result in an elevated breast cancer risk. The genes belonging to homologous recombination repair (HRR) pathway, such as X-ray Repair Cross Complementing Group 3 (*XRCC3*) have been extensively studied in association with various human cancers, mostly breast, lung or head and neck carcinomas [2-6].

XRCC3 Thr241Met variation (c. 722 C>T; rs 861539, Genbank accession number NT 026437) has been found to be associated with an increased formation of DNA adducts as well as with chromosomal deletions, and higher sensitivity to ionizing radiation and cross-linking agents [7].

A modest association between the homozygous variant genotype of the Thr241Met allele of *XRCC3* and breast cancer risk was first reported in a study in the United Kingdom [8]. However, most subsequent studies in Caucasian populations have not confirmed this association [9, 10].

Some but not all studies have found *XRCC3* Thr241Met to be related to an increased risk of breast cancer. Pooled analyses and meta-analyses show a small but significant increase in such risk [11-14].

In conclusion, *XRCC3* gene is a highly suspected candidate gene for cancer susceptibility. However, association studies on the *XRCC3* polymorphisms in cancer have shown conflicting results.

Therefore, in the present study, the association between breast cancer incidence in the population of Polish women and Thr241Met polymorphism of *XRCC3* gene was investigated.

Material and methods

Breast cancer patients

In the presented work, blood samples were collected from women (n = 760) with ductal breast carcinoma, treated from 2006 to 2011 at the Department of Oncology, Institute of Polish Mother's Memorial Hospital, Lodz, Poland. The age of the patients ranged from 41 to 84 years (mean age 48.4 ±10.11 years). No distant metastases were found in any of the patients at the time of treatment onset. The median follow-up of patients at the time of analysis was 39 months (the range: 2-71 months). The average tumour size was 22 mm (range 17-32 mm). All the tumours were graded by a method based on the criteria of Scarf-Bloom-Richardson. The characteristics of the patients are summarized in Table I. Blood samples from age-matched, cancer-free women (n = 760) served as controls (the mean age 43.41 ±18.22). An appropriate ethical approval was obtained from the Ethics Committee of the Institute of Polish Mother's Memorial Hospital, Lodz, Poland.

Table I. Characteristics of patients with breast cancer

BREAST CANCER	PATIENTS (N = 760)	
	N	%
Scarf-Bloom-Richardson stage		
I	206	27
II	500	66
III	54	7
Tumour size grade		
T1	95	13
T2	415	55
T3	190	25
T4	60	7
Lymph node status		
N0	350	46
N1	125	16
N2	150	20
N3	75	10
N4	60	8

PCR-RFLP analysis of XRCC3 genotype

XRCC3 gene polymorphism was determined by PCR-RFLP, using codon 241 primers (forward 5'-GCCTGGTGGTCATCGACTC-3' and reverse 5'-ACAGGGCTCTGGAAGGCACT GCTCAGCT-CACGCACC- 3'). The 25 µl PCR mixture contained 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. Thermal cycling conditions were the following: 94°C for 60 s, 56°C for 30 s and then 72°C for 40s, repeated in 30 cycles. The 552 bp amplified product was digested overnight with 5 U of *Nla*III (New England Biolabs, Ipswich, MA, USA) at 37°C. 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.

Statistical analysis

Genotype frequency deviations were assessed for each polymorphism, comparing Hardy-Weinberg equilibrium values with control values by the standard χ^2 test. Genotype frequencies in the study cases and controls were compared by the χ^2 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 II shows genotype distribution values of *XRCC3* Thr241Met polymorphism in breast cancer patients and controls. No statistically significant differences were observed in genotype frequencies of *XRCC3* Thr241Met polymorphisms between the control group and breast cancer patients.

Histological stages were evaluated in all the cases (n = 760); stage I – 206 cases, stage II – 500 cases and stage III – 54 cases. Stages II and III were taken to-

Table II. Genotype and allele frequency of *XRCC3* – Thr241Met polymorphisms in patients with breast cancer and controls

	BREAST CANCER PATIENTS (N = 760)		CONTROLS (N = 760)		OR (95% CI)[A]	P[B]
	NUMBER	(%)	NUMBER	(%)		
Thr/Thr	210	28	178	24	1.00 Ref.	
Thr/Met	370	49	366	48	0.85 (0.66-1.09)	0.243
Met/Met	180	23	216	28	0.70 (0.53-0.93)	0.184
Thr	730	48	722	47	1.00 Ref.	
Met	790	52	746	53	1.04 (0.90-1.21)	0.554

[A]Crude odds ratio (OR); 95% CI = confidence interval at 95%; [B]χ^2

Table III. Relation between genotypes and frequencies of *XRCC3* gene polymorphism, and the tumour stage in patients with breast cancer

STAGE[A]	BREAST CANCER PATIENTS (N = 760)		OR (95% CI)[B]	P[C]
	I (N = 206)	II + III (N = 554)		
XRCC3 Thr241Met				
Thr/Thr	42 (20)	168 (31)	1.00 Ref.	
Thr/Met	146 (71)	224 (40)	**2.61 (1.75-3.87)**	**< 0.0001**
Met/Met	18 (9)	162 (29)	**0.44 (0.24-0.80)**	**0.009**
Thr	230 (56)	560 (51)	1.00 Ref.	
Met	182 (44)	548 (49)	0.81 (0.64-1.01)	0.075

Data in boldface are statistically significant
[A]according to Scarf-Bloom-Richardson criteria; [B]crude odds ratio (OR), 95% CI – confidence interval at 95%, [C]χ^2

gether for statistical analysis (see Table III). Some correlation was observed between the genotypes of *XRCC3*-Thr241Met polymorphisms and breast cancer invasiveness. A statistically significant increase was observed regarding Thr/Met heterozygotes in stage I patients according to Scarf-Bloom-Richardson classification.

The distribution of genotypes and the frequency of alleles in patients with (N+) and without (N–) lymph node metastases are presented in Table IV. A tendency for a decreased risk of breast cancer was observed with the occurrence of Met/Met genotype and Met allele of *XRCC3* polymorphism. That decrease was statistically significant ($p < 0.05$) (see Table IV). There were no differences either in the distribution of genotypes or the frequency of alleles in the group of patients with different tumour size (Table V).

Discussion

XRCC3 plays an important role in the repair of DNA double-strand breaks (DSBs) by homologous recombination. DSBs may contribute to the pathogenesis of various cancers and variability in DNA repair genes. XRCC2 and XRCC3 proteins are structurally and functionally related to RAD51, which plays

Table IV. Relation between genotypes and frequencies of *XRCC3* gene polymorphism, and the node status

	BREAST CANCER PATIENTS (N = 760) NODE STATUS[A]		OR (95% CI)[B]	P[C]
	N+ (N = 410)	N– (N = 350)		
XRCC3 Thr241Met				
Thr/Thr	142 (35)	104 (29)	1.00 Ref.	
Thr/Met	168 (42)	114 (33)	1.07 (0.76-1.52)	0.729
Met/Met	100 (23)	132 (38)	**0.55 (0.38-0.79)**	**0.0019**
Thr	452 (56)	322 (46)	1.00 Ref.	
Met	368 (44)	378 (54)	**0.69 (0.56-0.84)**	**0.00048**

Data in boldface are statistically significant
[A]N– (node negative) vs. N+ (node positive), [B]crude odds ratio (OR), 95% CI – confidence interval at 95%, [C]χ^2

Table V. Relation between genotypes and frequencies of *XRCC3* gene polymorphism, and the tumour size (T2 vs. T3 + T4)

	BREAST CANCER PATIENTS (N = 760)		OR (95% CI)[A]	P[B]
	TUMOUR SIZE			
	T3 + T4 (N = 250)	T1 + T2 (N = 510)		
XRCC3 Thr241Met				
Thr/Thr	85 (34)	184 (36)	1.00 Ref.	
Thr/Met	104 (43)	196 (39)	1.14 (0.81-1.63)	0.492
Met/Met	61 (23)	130 (25)	1.01 (0.68-1.51)	1.000
Thr	274 (55)	564 (57)	1.00 Ref.	
Met	226 (45)	456 (43)	1.02 (0.82-1.26)	0.887

[A]crude odds ratio (OR), 95% CI – confidence interval at 95%, [B]$\chi 2$

an important role in the homologous recombination, the process being frequently involved in cancer transformation [8]. *RAD51*, *XRCC2* and *XRCC3* gene are highly polymorphic.

The Thr241Met substitution is the most thoroughly investigated polymorphism in *XRCC3* due to a (C>T) transition at exon 7 (*XRCC3*-18067C>T, rs861539).

In the presented study we investigated whether single nucleotide polymorphisms (SNPs) *XRCC3*-Thr241Met were associated with the risk of breast cancer in Polish women.

The study was performed on an ethnically homogenous population, which may improve our knowledge as to what extent the genotype-phenotype relationship variations are population-related.

In the reported study, the investigated polymorphism, Thr241Met of *XRCC3* gene, was associated with breast carcinoma progression. Thr241Met heterozygote was associated with an increased risk of stage I breast cancer.

However, other literature data were also found [2, 10, 15]. No significant associations were observed between the Thr241Met and breast cancer in Cypriot women [15]. Moreover, in Caucasian women genotypes in *XRCC3* were not associated with an increased risk of developing breast cancer [16]. DNA repair polymorphism of *XRCC3* was correlated with histopathological characteristics and hormone receptors in a group of Brazilian women with breast cancer. No statistically significant association was found between gene polymorphism and hormone receptor status. Thr241Met polymorphism was not associated with breast cancer development in these women [17].

In the Polish population, Thr241Met genotype of *XRCC3* polymorphism slightly increased the risk of local metastasis in breast cancer patients [2, 10]. The combined Thr241Met-XRCC3/135G/C-RAD51 genotype decreased the risk of breast cancer occurrence [10].

Similarly to our observation, recent reports demonstrate that *XRCC3* Thr241Met allele seems associated with an elevated breast cancer risk in non-Chinese subjects [14]. He *et al.* indicated that Thr241Met polymorphism may be associated with an increased breast cancer risk [18]. Costa *et al.* suggested *XRCC1* Arg399Gln and *XRCC3* Thr241Met DNA repair polymorphisms to be important biomarkers to sporadic breast cancer susceptibility [12].

In conclusion, the reported study is another evidence for the significance of Thr241Met genotype in breast carcinoma staging. The obtained data show that polymorphism of *XRCC3* gene may be associated with the risk of breast carcinoma occurrence. On the other hand, a protective effect was observed of Thr241Met polymorphism in patients without (N–) lymph node metastasis.

Finally, it is postulated that this polymorphism may be used as a predictive factor for breast cancer in the Polish female population. Further studies conducted on a larger group are suggested to clarify this point.

References

1. Parkin DM, Bray FI, Devesa SS. Cancer burden in the year 2000. The global picture. Eur J Cancer 2001; 37: 4-66.
2. Sobczuk A, Romanowicz-Makowska H, Fiks T, et al. XRCC1 and XRCC3 DNA repair gene polymorphisms in breast cancer women from the Lodz region of Poland. Pol J Pathol 2009; 60: 76-80.
3. Romanowicz-Makowska H, Smolarz B, Zadrozny M, et al. Single nucleotide polymorphisms in the homologous recombination repair genes and breast cancer risk in Polish women. Tohoku J Exp Med 2011; 224: 201-208.
4. Santos RA, Teixeira AC, Mayorano MB, et al. DNA repair genes XRCC1 and XRCC3 polymorphisms and their relationship with the level of micronuclei in breast cancer patients. Genet Mol Biol 2010; 33: 637-640.
5. Metro G, Chiari R, Mare M, et al. Carboplatin plus pemetrexed for platinum-pretreated, advanced non-small cell lung cancer: a retrospective study with pharmacogenetic evaluation. Cancer Chemother Pharmacol 2011; 68: 1405-1412.
6. Pratesi N, Mangoni M, Mancini I, et al. Association between single nucleotide polymorphisms in the XRCC1 and RAD51 genes and clinical radiosensitivity in head and neck cancer. Radiother Oncol 2011; 99: 356-361.
7. Au W, Salama S, Sierra-Torres C. Functional characterization of polymorphisms in DNA repair genes using cytogenetic chal-

lenge assays. Environ. Health Perspect 2003; 111: 1843-1850.
8. Kuschel B, Auranen A, McBride S, et al. Variants in DNA double-strand break repair genes and breast cancer susceptibility. Hum Mol Genet 2002; 11: 1399-1407.
9. Sterpone S, Mastellone V, Padua L, et al. Single-nucleotide polymorphisms in BER and HRR genes, XRCC1 haplotypes and breast cancer risk in Caucasian women. J Cancer Res Clin Oncol 2010; 136: 631-636.
10. Krupa R, Synowiec E, Pawlowska E, et al. Polymorphism of the homologous recombination repair genes RAD51 and XRCC3 in breast cancer. Exp Mol Pathol 2009; 87: 32-35.
11. Thyagarajan B, Anderson KE, Folsom AR, et al. No association between XRCC1 and XRCC3 gene polymorphisms and breast cancer risk: Iowa Women's Health Study. Cancer Detect Prev 2006; 30: 313-321.
12. Costa S, Pinto D, Pereira D, Rodrigues H, et al. DNA repair polymorphisms might contribute differentially on familial and sporadic breast cancer susceptibility: a study on a Portuguese population. Breast Cancer Res Treat 2007; 103: 209-217.
13. Sangrajrang S, Schmezer P, Burkholder I, et al. The XRCC3 Thr241Met polymorphism and breast cancer risk: a case control study in a Thai population. Biomarkers 2007; 12: 523-532.
14. Economopoulos KP, Sergentanis TN. XRCC3 Thr241Met polymorphism and breast cancer risk: a meta-analysis. Breast Cancer Res Treat 2010; 121: 439-443.
15. Loizidou MA, Michael T, Neuhausen SL, et al. Genetic polymorphisms in the DNA repair genes XRCC1, XRCC2 and XRCC3 and risk of breast cancer in Cyprus. Breast Cancer Res Treat 2008; 112: 575-579.
16. Sterpone S, Mastellone V, Padua L, et al. Single-nucleotide polymorphisms in BER and HRR genes, XRCC1 haplotypes and breast cancer risk in Caucasian women. J Cancer Res Clin Oncol 2010; 136: 631-636.
17. Dufloth RM, Arruda A, Heinrich JK, et al. The investigation of DNA repair polymorphisms with histopathological characteristics and hormone receptors in a group of Brazilian women with breast cancer. Genet Mol Res 2008; 7: 574-582.
18. He XF, Wei W, Su J, et al. Association between the XRCC3 polymorphisms and breast cancer risk: meta-analysis based on case-control studies. Mol Biol Rep 2012; 39: 5125-5134.

Address for correspondence

Hanna Romanowicz-Makowska
Laboratory of Molecular Genetics
Department of Pathology
Institute of Polish Mother's Memorial Hospital
ul. Rzgowska 281/289
93-338 Łódź
phone +48 42 271 20 71
e-mail: smolbea@wp.pl