XRCC1 and *XRCC3* DNA repair gene polymorphisms in breast cancer women from the Lodz region of Poland

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Aim: Genetic polymorphism in *XRCC1* and *XRCC3* genes may influence DNA repair capacity and, in turn, confer predisposition to breast cancer.
Material and methods: In the present work the distribution of genotypes and frequency of alleles of the *Arg194Trp* and *Arg399Gln* polymorphism of *XRCC1* and *Trp241Met* polymorphism in *XRCC3* in breast cancer women were analysed. Blood samples were obtained from 150 women with breast cancer and controls (n = 106). The polymorphisms were determined by PCR-RFLP methods.
Results: No association between *XRCC1 Arg399Gln* and *Arg194Trp* genotype and breast cancer risk was observed. The distribution of the genotypes of the *Trp241Met* polymorphism of *XRCC3* in both controls and patients did not differ significantly ($p > 0.05$) from those predicted by the Hardy-Weinberg distribution. There were no significant differences ($p > 0.05$) in genotype distributions and allele frequencies between subgroups assigned to histological stage.
Conclusion: The results suggest that the *Arg194Trp* and *Arg399Gln* polymorphism of the *XRCC1* gene as well as *Trp241Met* polymorphism in *XRCC3* may not be linked with appearance and development of breast cancer.

Key words: XRCC1, XRCC3, gene polymorphism, breast cancer

Introduction

Breast cancer is a cancer of breast tissue. Worldwide, it is the most common form of cancer in females, affecting approximately one out of nine to thirteen women who reach age ninety at some stage of their life in the Western world. It is (after lung cancer) the second most fatal cancer in women, and the number of cases has significantly increased since the 1970s, a phenomenon partly blamed on modern lifestyles in the Western world [1, 2].

Repair of DNA damage is under genetic control, and DNA repair genes may play a key role in maintaining genome integrity and preventing cancer development. Polymorphisms in DNA repair genes resulting in variation of DNA repair efficiency may therefore be associated with cancer risk [3-5].

Many DNA repair genes have been identified: they are involved in several rare recessive inherited DNA repair syndromes such as ataxia-telangiectasia, Fanconi's anaemia, Bloom's syndrome, and xeroderma pigmentosum, which are characterised by hypersensitivity to carcinogens and high risk of cancer. Although genetic variants of these genes at one or more loci are likely to be associated with only moderate changes in cancer risk, they are prevalent in the population and may contribute to the overall population risk of cancer [6-12]. There are five major DNA repair mechanisms: base excision, nucleotide excision, mismatch repair, photoreactivation (utilizing near UV light as well as an appropriate enzyme system) and recombination repair [13-15].

Because BER, HRR and NER play critical roles in repairing various types of DNA damage, combined

genetic variants of these three repair pathways may contribute to a greater risk of breast cancer [8, 10, 11].

Common polymorphisms in DNA repair genes such as *XRCC1*, *XRCC3* and *ERCC4/XPF*, may alter protein function and an individual's capacity to repair damaged DNA; deficits in repair capacity may lead to genetic instability and breast cancer development [16-18]. Several studies have been conducted to assess the relationship between polymorphism R399Q of the *XRCC1* gene and Arg415Gln of *ERCC4/XPF* and the risk of breast cancer [10, 11, 19-22]. But the results are fairly inconsistent, and no conclusions can be drawn at present.

Polymorphism of *XRCC3* may result in reduced DNA repair capacity, but direct functional research evidence is absent, and epidemiological research results are inconclusive at present. A protective effect of *XRCC3* 241Met allele on human cancer is plausible, although little is known about the biochemical properties and biological functions of *XRCC3* protein and the functional changes associated with this polymorphism [18, 11]. *XRCC3* was shown to interact directly with HsRad51 and, as with Rad55 and Rad57 in yeast, may cooperate with HsRad51 during recombination repair.

In our earlier study we suggested that the G/C polymorphism of the RAD51 gene may not be directly involved in the development and/or progression of breast cancer [23, 24]. In this study, we hypothesized that there exists an association between breast cancer and three genetic polymorphisms in DNA repair genes *XRCC1* and *XRCC3*.

Materials and methods

Patients

Blood samples were obtained from 150 post-menopausal women with node-negative and node-positive ductal breast carcinoma. No distant metastases were found in patients at the time of treatment. The patients ranged in age from 54 to 82 years (median age 58 years). The average tumour size was 20 mm (range 17-32 mm). All tumours were graded by a method based on the criteria of Scarff-Bloom-Richardson. There were 43 tumours of grade I, 76 of grade II and 31 of grade III in total. Blood samples (n = 106) from age-matched healthy women served as controls.

DNA isolation

Genomic DNA was isolated from 200 μl of whole blood, using QIAamp DNA Blood Mini Kits (Qiagen GmbH, Hilden, Germany).

PCR-RFLP genotyping assays

Genotypic analyses of the *XRCC1* gene were carried out by multiplex PCR-RFLP, using primers for codons 399 (5'-TTGTGCTTTCTCTGTGTCCA-3' and 5'-TCCTCCAGCCTTTTCTGATA-3') and 194 (5'-GCCCCGTCCCAGGTA-3' and 5'-AGCCCCA-AGACCCTTTCACT-3'), which generate a fragment of 615 and of 491 bp. Briefly, PCR was performed in 25 μl reaction buffer containing 12.5 pmol of each primer, 0.2 mmol/l of dNTPs, 3 mmol/l of $MgCl_2$, about 100 ng of DNA and 1 IU of Taq DNA polymerase. The PCR products were digested overnight with 10 IU of *Msp*I at 37°C.

The wild-type Arg allele for codon 194 is identified by the presence of a 293 bp band, and the mutant Trp allele by the presence of a 313 bp band (indicative of the absence of the *Msp*I cutting site). For codon 399, the presence of two bands of 375 and 240 bp, respectively, identifies the wild-type Arg allele, while the uncut 615 bp band identifies the mutant Gln allele (indicative of absence of the *Msp*I cutting site).

Polymorphism of the *XRCC3* gene was determined by PCR-RFLP, using codon 241 primers (5'-GCCTGGTGGTCATCGACTC-3' and 5'-AC-AGGGCTCTGGAAGGCACT GCTCAGCTCA-CGCACC-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 IU of Taq DNA polymerase. The 552 bp amplified product was digested overnight with 5 IU of *Nla*III 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

The allelic frequencies were estimated by gene counting and genotypes were scored. The observed numbers of each *XRCC1* and *XRCC3* genotype were compared with that expected for a population in Hardy-Weinberg equilibrium by using a χ^2 test. The significance of the differences of observed alleles and genotypes between groups was tested using the χ^2 analysis. P-values < 0.05 were considered to be significant.

Results

From the PCR analysis, all the patients and controls were divided into three genotypes of Arg399Gln polymorphism of the *XRCC1* gene Arg/Arg, Arg/Gln and Gln/Gln. Table I shows Arg and Gln genotype distribution between breast cancer patients and controls. Both distributions did not differ significantly ($p > 0.05$) from those predicted by the Hardy-Weinberg distribution. Additionally there were no differences in the frequencies of the Arg and Gln alleles between patients and controls.

Distributions of the Arg/Arg, Arg/Trp and Trp/Trp genotypes of the *XRCC1* gene as well as the frequencies of the Arg and Trp alleles for breast cancer subjects and controls are displayed in Table II. It can be seen from the table that there were no significant differences between these two groups in both genotype distribution and allele frequencies ($p > 0.05$).

Dependencies of the distribution of genotypes and frequencies of Thr and Met alleles of the *XRCC3* investigated polymorphism in patients with breast cancer and controls are displayed in Table III. There were no significant differences between distributions of genotypes in patients and controls and the distribution predicted by Hardy-Weinberg equilibrium.

Dependencies of the distribution of genotypes and frequencies of alleles of Arg194Trp and Arg399Gln polymorphism of the *XRCC1* gene and Trp241Met polymorphism of the *XRCC3* gene investigated polymorphism at the tumour stage evaluated according to Bloom-Scarf-Richardson criteria of patients with breast cancer was investigated. There were no significant differences between distributions of genotypes in subgroups assigned to histological stage and

Table I. Distribution of Arg/Arg, Arg/Gln, and Gln/Gln genotypes and frequencies of the Arg and Gln alleles of Arg399Gln of *XRCC1* polymorphism in patients with breast cancer and controls

	Breast cancer (n = 150)		Control (n = 106)	
	number	frequency	number	frequency
Arg/Arg	34	0.23	24	0.23
Arg/Gln	68	0.45	44	0.42
Gln/Gln	48	0.32	38	0.36
χ^2	3.657[a]		0.572[a]	
Arg allele	136	0.45[b]	92	0.43
Gln allele	164	0.55[b]	120	0.57

[a]p > 0.05 compared with Hardy-Weinberg equilibrium; [b]p > 0.05 compared with control

Table II. Distribution of Arg/Arg, Arg/Trp, and Trp/Trp genotypes and frequencies of the Arg and Trp alleles of Arg194Trp of *XRCC1* polymorphism in patients with breast cancer and controls

	Breast cancer (n = 150)		Control (n = 106)	
	number	frequency	number	frequency
Arg/Arg	36	0.24	20	0.19
Arg/Trp	70	0.47	52	0.49
Trp/Trp	44	0.29	34	0.32
χ^2	3.436[a]		0.652[a]	
Allele Arg	142	0.47[b]	92	0.43
Allele Gln	158	0.53[b]	120	0.57

[a]p > 0.05 compared with Hardy-Weinberg equilibrium; [b]p > 0.05 compared with control

Table III. Distribution of Trp/Trp, Trp/Met, and Met/Met genotypes and frequencies of the Trp and Met alleles of *XRCC3* polymorphism in patients with breast cancer and controls

	Breast cancer (n = 150)		Control (n = 106)	
	number	frequency	number	frequency
Trp/Trp	29	0.19	24	0.23
Trp/Met	71	0.47	50	0.47
Met/Met	50	0.33	32	0.30
χ^2	3.457[a]		0.567[a]	
Trp allele	129	0.43[b]	98	0.46
Met allele	171	0.57[b]	114	0.54

[a]p > 0.05 compared with Hardy-Weinberg equilibrium; [b]p > 0.05 compared with control

the distribution predicted by Hardy-Weinberg equilibrium ($p > 0.05$). There were no differences in frequencies of any of the alleles between subgroups either ($p > 0.05$).

Discussion

Various types of DNA damage are repaired through multiple repair pathways in which a number of proteins play a role. The *XRCC1* gene is mapped at human chromosome 19q13.2-13.3 and XRCC1 protein is an important component of the base excision repair pathway, which fixes base damage and DNA single strand breaks caused by ionizing radiation and alkylating agents. XRCC3 is one of five identified paralogues of the strand-exchange protein RAD51 in humans and functions through complex interactions with other relevant proteins to repair double strand breaks and maintain genome integrity in multiple centrosomes and abnormal recombination.

Because BER, HRR and NER play critical roles in repairing various types of DNA damage, combined genetic variants of these three repair pathways may contribute to a greater risk of breast cancer. DNA repair gene variability could contribute to the level of the protein's biosynthesis. In view of the potential significant role of XRCC1 and XRCC3 for tumour development, it is important to know whether these polymorphisms can account for the development and/or progression of breast cancer [25, 26].

The XRCC1 protein plays an important role in base excision repair (BER); after excision of a damaged base, it stimulates endonuclease action and acts as a scaffold in the subsequent restoration of the site [16]. Three polymorphisms in *XRCC1* (R194W, R399Q R280H) were investigated; only R194W polymorphism was associated with breast cancer risk in African Americans and Caucasians [10, 22]. Most of the published R194W studies reported a reduced risk of cancer associated with the W allele [10, 22]. A second *XRCC1* polymorphism (R399Q) has also been well studied; however, the results suggested associations in different directions for different cancers: decreased risk for non-melanoma skin carcinoma [27], oesophageal cancer [28] and bladder cancer [29]; increased risk for stomach cancer [30] and breast cancer [10].

XRCC3 participates in homologous recombination repair of DNA double strand breaks and cross-links. It is a member of an emerging family of Rad-51-related proteins that may take part in homologous recombination to maintain chromosome stability and to repair DNA damage [13]. XRCC3 deficient cells were found to be unable to form Rad51 foci after radiation damage and demonstrated genetic instability and increased sensitivity to DNA-damaging agents. The Thr241Met substitution in XRCC3 is due to a ($C \rightarrow T$) transition at exon 7 and is a non-conservative change but does not reside in the ATP-binding domains, which are the only functional domains that have been identified in the protein at this time [11].

In this work conducted on 150 ductal breast carcinoma patients we did not find any correlation between Arg194Trp, Arg399Gln and Thr241Met polymorphism and occurrence of cancer. Moreover we did not detect any significant difference between genotypes of node positive and node negative patients, which suggests a lack of association between polymorphisms and breast cancer invasiveness.

Our study implies that it is possible that the Arg194Trp, Arg399Gln polymorphism of *XRCC1* and Thr241Met polymorphism of the *XRCC3* gene may not be directly involved in the development and/or progression of breast cancer, but further research, conducted on a larger population, is needed to clarify this point.

References

1. McGuire W, Clark GM. Prognostic factors and treatment decisions in axillary node-negative breast cancer. N Engl J Med 1992; 326: 1756-1761.
2. Ravaioli A, Bagli L, Zucchini A, Monti F. Prognosis and prediction of response in breast cancer. The current role of the main biological markers. Cell Proliferation 1998; 31: 113-126.
3. Khanna KK, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. Nat Genet 2001; 27: 247-254.
4. Aquilina G, Bignami M. Mismatch repair in correction of replication errors and processing of DNA damage. J Cell Physiol 2001; 187: 145-154.
5. Lengauer C, Kinzler K, Vogelstain B. Genetic instabilities in human cancers. Nature 1998; 396: 643-649.
6. Shi Q, Wang Li-E, Bondy M, et al. Reduced DNA repair of benzo [a] pyrene diol epoxide-induced adducts and common XPD polymorphisms in breast cancer patients. Carcinogenesis 2004; 25: 1695-1700.
7. Shen M, Hung RJ, Brennan P, et al. Polymorphisms of DNA Repair Genes XRCC1, XRCC3, XPD, interaction with environmental exposures, and bladder cancer risk in a case-control study in northern Italy. Cancer Epidemiol Biomark Prev 2003; 12: 1234-1240.
8. Webb PM, Hopper JL, Newman B, et al. Double-strand break repair gene polymorphisms and risk of breast or ovarian cancer. Cancer Epidemiol Biomark Prev 2005, 14: 319-323.
9. Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA Repair Genes and Associations with Cancer Risk. Cancer Epidemiol. Biomark. Prev. 2002; 11: 1513-1530.
10. Duell EJ, Milikan RC, Pitman GS, et al. Polymorphisms in the DNA repair gene XRCC1 and breast cancer. Cancer Epidemiol Biomark Prev 2001; 10: 217-222.
11. Smith TR, Levine EA, Perrier ND, et al. DNA-Repair Genetic Polymorphism and Breast Cancer Risk. Cancer Epidemiol Biomark Prev 2003; 12: 1200-1204.
12. Fu YP, Yu JC, Cheng TC, et al. Breast Cancer risk associated with genotypic polymorphism of the nonhomologous endjoining genes. Cancer Res 2003; 63: 2440-2446.

13. Vispe S, Yung TM, Ritchot J, et al. A cellular defense pathway regulating transcription through poly (ADP-ribosyl) ation in response to DNA damage. Proc Natl Acad Sci U S A 2000; 97: 9886-9891.
14. Roberts RJ, Cheng X. Base flipping. Annu Rev Biochem 1998; 67: 181-198.
15. Sobol RW, Horton JK, Kuhn R, et al. Requirement of mammalian DNA polymerase-β in base excision repair. Nature 1996; 379: 183-186.
16. Thompson LH, West MG: XRCC1 keeps DNA from getting stranded. Mutat Res 2000; 459: 1-18.
17. Bessho T, Sancar A, Thompson LH, Thelen MP. Reconstitution of human excision nuclease with recombinant XPF-ERCC1 complex. J Biol Chem 1997; 272: 3833-3837.
18. Bishop DK, Ear U, Bhattacharyya A, et al. Xrcc3 is required for assembly of Rad51 complexes in vivo. J Biol Chem 1998; 273: 21482-21488.
19. Zhai X, Liu J, Hu Z, et al. Polymorphisms of ADPRT Val762Ala and XRCC1 Arg399Glu and risk of breast cancer in Chinese women: a case control analysis. Oncol Rep 2006; 15: 247-252.
20. Metsola K, Kataja V, Sillanpaa P, et al. XRCC1 and XPD genetic polymorphisms, smoking and breast cancer risk in a Finnish case-control study. Breast Cancer Res 2005; 7: 987-997.
21. 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.
22. Chacko P, Rajan B, Joseph T, et al. Polymorphisms in DNA repair gene XRCC1 and increased genetic susceptibility to breast cancer. Breast Cancer Res Treat 2005; 89: 15-21.
23. Romanowicz-Makowska H, Smolarz B, Kulig A. The G/C polymorphism of RAD51 gene in breast cancer. Pol Merkuriusz Lek 2006; 21: 55-58.
24. Romanowicz-Makowska H, Smolarz B, Zadrozny M, Kulig A. Analysis of RAD51 polymorphism and BRCA1 mutations in Polish women with breast cancer. Exp Oncol 2006; 28: 156-159.
25. Spencer CC. Human polymorphism around recombination hotspots. Biochem Soc Trans 2006; 34: 535-536.
26. Chang-Claude J, Popanda O, Tan XL, et al. Association between polymorphisms in the DNA repair genes, XRCC1, APE1, and XPD and acute side effects of radiotherapy in breast cancer patients. Clin Cancer Res 2005; 11: 4802-4809.
27. Nelson H, Kelsey K, Mott L, Karagas MR. The XRCC1 Arg399Gln polymorphism, sunburn, and non-melanoma skin cancer: evidence of gene-enviroment interaction. Cancer Res 2002; 62: 152-155.
28. Lee JM, Lee YC, Yang SY, et al. Genetic polymorphism of XRCC1 and risk of the esophageal cancer. Int J Cancer 2001; 95: 240-246.
29. Stern MC, Umbach DM, van Gills CH, Lunn RM. DNA repair gene XRCC1 polymorphism, smoking and bladder cancer risk. Cancer Epidemiol. Biomark Prev 2001; 10: 125-131.
30. Shen H, Xu Y, Qian Y, et al. Polymorphism of the DNA repair gene XRCC1 and risk of gastric cancer in a Chinese population. Int J Cancer 2000; 88: 601-606.

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