**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

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**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'-TTGTGCTTTTCTCTGTGCA-3' and 5'-TTCTGCCAGTCTTTCTGATA-3') and 194 (5'-GCCCGCTCCAGGTA-3' and 5'-AGCCCCAGACCTTTTACT-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 MgCl2, about 100 ng of DNA and 1 IU of Taq DNA polymerase. The PCR products were digested overnight with 10 IU of MspI 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 MspI 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 MspI cutting site).

Polymorphism of the XRCC3 gene was determined by PCR-RFLP, using codon 241 primers (5'-GCTTGGTGCTCATGACTC-3' and 5'-AGGGCCTCTGGAAGGCACT GCTCAGCTCAA-CGGACC-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 MgCl2 and 1 IU of Taq DNA polymerase. The 532 bp amplified product was digested overnight with 5 IU of NlaIII at 37°C. The wild-type allele Thr was identified by the presence of two 239 and 315 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 $\chi^2$ test. The significance of the differences of observed alleles and genotypes between groups was tested using the $\chi^2$ 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 and frequencies of Thr and Met alleles of the XRCC3 investigated polymorphism in patients with breast cancer and controls.

### 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

<table>
<thead>
<tr>
<th></th>
<th>BREAST CANCER (N = 150)</th>
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<th>CONTROL (N = 106)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>NUMBER</td>
<td>FREQUENCY</td>
<td>NUMBER</td>
<td>FREQUENCY</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>34</td>
<td>0.23</td>
<td>24</td>
<td>0.23</td>
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<td>Arg/Gln</td>
<td>68</td>
<td>0.45</td>
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<td>0.42</td>
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<td>Gln/Gln</td>
<td>48</td>
<td>0.32</td>
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<td>0.36</td>
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<tr>
<td>χ²</td>
<td>3.657ᵃ</td>
<td></td>
<td>0.572ᵇ</td>
<td></td>
</tr>
<tr>
<td>Arg allele</td>
<td>136</td>
<td>0.45ᵇ</td>
<td>92</td>
<td>0.43</td>
</tr>
<tr>
<td>Gln allele</td>
<td>164</td>
<td>0.55ᵇ</td>
<td>120</td>
<td>0.57ᵇ</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
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<td>NUMBER</td>
<td>FREQUENCY</td>
<td>NUMBER</td>
<td>FREQUENCY</td>
</tr>
<tr>
<td>Arg/Arg</td>
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<td>20</td>
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<tr>
<td>Arg/Trp</td>
<td>70</td>
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<td>52</td>
<td>0.49</td>
</tr>
<tr>
<td>Trp/Trp</td>
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<td>0.29</td>
<td>34</td>
<td>0.32</td>
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<tr>
<td>χ²</td>
<td>3.436ᵃ</td>
<td></td>
<td>0.652ᵇ</td>
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<tr>
<td>Allele Arg</td>
<td>142</td>
<td>0.47ᵇ</td>
<td>92</td>
<td>0.43</td>
</tr>
<tr>
<td>Allele Gln</td>
<td>158</td>
<td>0.55ᵇ</td>
<td>120</td>
<td>0.57ᵇ</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
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<th>BREAST CANCER (N = 150)</th>
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<th>CONTROL (N = 106)</th>
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<td></td>
<td>NUMBER</td>
<td>FREQUENCY</td>
<td>NUMBER</td>
<td>FREQUENCY</td>
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<tr>
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<td>71</td>
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<tr>
<td>Met/Met</td>
<td>50</td>
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<tr>
<td>χ²</td>
<td>3.457ᵃ</td>
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<td>0.567ᵇ</td>
<td></td>
</tr>
<tr>
<td>Trp allele</td>
<td>129</td>
<td>0.43ᵇ</td>
<td>98</td>
<td>0.46</td>
</tr>
<tr>
<td>Met allele</td>
<td>171</td>
<td>0.57ᵇ</td>
<td>114</td>
<td>0.54</td>
</tr>
</tbody>
</table>

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 → 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**


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