SINGLE NUCLEOTIDE POLYMORPHISM (SNP) Thr241Met in the XRCC3 gene and breast cancer risk in Polish women

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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.

PCR-RFLP analysis of XRCC3 genotype

XRCC3 gene polymorphism was determined by PCR-RFLP, using codon 241 primers (forward 5’- GCCCTGGTGTCATCGACTC-3’ and reverse 5’-ACAGGGCTCTCGAGGGCATGCTCAGCT-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 MgCl2 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 40 s, repeated in 30 cycles. The 552 bp amplified product was digested overnight with 5 U of NlaIII (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 χ2 test. Genotype frequencies in the study cases and controls were compared by the χ2 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-
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 tumor 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...
an important role in the homologous recombination, the process being frequently involved in cancer transformation [8]. \( \text{RAD51} \), \( \text{XRCC2} \) and \( \text{XRCC3} \) gene are highly polymorphic.

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

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

The study was performed on an ethnically homogeneous 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 \( \text{XRCC3} \) 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 \( \text{XRCC3} \) were not associated with an increased risk of developing breast cancer [16]. DNA repair polymorphism of \( \text{XRCC3} \) 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 \( \text{XRCC3} \) polymorphism slightly increased the risk of local metastasis in breast cancer patients [2, 10]. The combined Thr241Met-\( \text{XRCC3} \)/\( \text{RAD51} \) genotype decreased the risk of breast cancer occurrence [10].

Similarly to our observation, recent reports demonstrate that \( \text{XRCC3} \) 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 \( \text{XRCC1} \) Arg399Gln and \( \text{XRCC3} \) 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 \( \text{XRCC3} \) 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

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Table V.

<table>
<thead>
<tr>
<th>TUMOUR SIZE</th>
<th>BREAST CANCER PATIENTS (N = 760)</th>
<th>OR (95% CI)(^a)</th>
<th>p(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{T3 + T4 (N = 250)} )</td>
<td>( \text{T1 + T2 (N = 510)} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{XRCC3 Thr241Met} )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr/Thr</td>
<td>85 (34)</td>
<td>184 (36)</td>
<td>1.00 Ref.</td>
</tr>
<tr>
<td>Thr/Met</td>
<td>104 (43)</td>
<td>196 (39)</td>
<td>1.14 (0.81-1.63)</td>
</tr>
<tr>
<td>Met/Met</td>
<td>61 (23)</td>
<td>130 (25)</td>
<td>1.01 (0.68-1.51)</td>
</tr>
<tr>
<td>Thr</td>
<td>274 (55)</td>
<td>564 (57)</td>
<td>1.00 Ref.</td>
</tr>
<tr>
<td>Met</td>
<td>226 (45)</td>
<td>456 (43)</td>
<td>1.02 (0.82-1.26)</td>
</tr>
</tbody>
</table>

\(^a\) Crude odds ratio (OR), 95% CI – confidence interval at 95%, \(^b\) \( \chi^2 \)

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