Null Results in Brief

Genetic Polymorphisms in the DNA Double-Strand Break Repair Genes XRCC3, XRCC2, and NBS1 Are Not Associated with Acute Side Effects of Radiotherapy in Breast Cancer Patients

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Introduction

Clinical sensitivity to ionizing radiation varies considerably among patients, and radiation-induced adverse effects developing in normal tissue can be therapy limiting in >10% of patients (1). Ionizing radiation induces both DNA single-strand breaks and double-strand breaks (DSB), with the DSBs generally considered the lethal event (2). Several syndromes associated with increased radiosensitivity are caused by deficiencies in genes of DSB repair, leading to the hypothesis that the individual repair capacity for these lesions should be an important determinant of individual radiosensitivity (3, 4). Here, three polymorphisms in genes involved in homologous recombination (XRCC3 Thr241Met, XRCC2 Arg188His, and NBS1 Glu185Gln) with potential functional effects (5-8) were evaluated for a possible association with the risk of developing acute skin reactions following radiotherapy in a prospective epidemiologic study.

Materials and Methods

Study subjects included female breast cancer patients receiving primary radiotherapy after breast-conserving surgery as reported (ref. 9; reference no. 37/98 of the ethical committee of the University of Heidelberg). All the patients were given a typical breast radiation treatment with an average biologically effective radiation dose of 54.0 ± 4.8 Gy. Clinical radiation reaction developing in the skin within the radiation field of the breast was documented at regular time intervals during treatment, and the severity of acute side effects was assessed using a classification system based on the Common Toxicity Criteria of the U.S. NIH (10). Seventy-seven of the 446 participants presented with increased acute toxicity (at least one moist desquamation or interruption of radiotherapy due to toxicity) by the end of treatment (9, 11).

DNA extraction, analysis of the XRCC3 Thr241Met polymorphism (rs861539), and quality control was as described (12). The XRCC2 Arg188His (rs3218536) and the NBS1 Glu185Gln (rs1805794) polymorphisms were measured as other variants in (12) by melting curve analysis of sequence-specific hybridization probes (LightCycler, Roche Diagnostics, Mannheim, Germany). For XRCC2 Arg188His, PCR primers were 5’-TGGATATGCTCCGGCTAGTTA-3’ and 5’-CTGCCATGCTCCTAAGGATTT-3’, and probes were 5’-CTGTGAAATGAGCTGTTTFT-3’ and 5’-LCreGGAATGAGGACTGTTTFT-3’ respectively. Primers were 5’-TTATATGAGTAACGCGCTCT-3’ and 5’-AAACCTTCATTTAATAATCCGAA-3’, and probes were 5’-LCreGGAATGAGGACTGTTTFT-3’ and 5’-TGAATTCCTGAAACGATGCAGTCAGTTC-3’ (Tib Molbiol, Berlin, Germany). Genotype information was incomplete for two samples because of an inadequate amount of DNA.

Occurrence of acute skin toxicity was analyzed using Cox proportional hazards model in relation to biologically effective radiation dose instead of time in days during radiotherapy, thereby adjusting for differences in radiation dose when acute skin toxicity occurred and for the total dose received (13). As possible confounders, differences by treating hospitals and body mass index were included in all models (9).

Results

Frequencies of rare alleles were 0.41, 0.07, and 0.32 for XRCC3 Thr241Met, XRCC2 Arg188His, and NBS1 Glu185Gln, respectively, consistent with published reports (0.39, XRCC3; 0.06, XRCC2; and 0.32, NBS1) in European populations (8, 12, 14). All genotype distributions were in Hardy-Weinberg equilibrium. Overall, we did not observe a significant association between the genetic polymorphisms investigated and the risk of acute skin toxicity after radiotherapy (Table 1). Analysis yielded differences in the associations between XRCC3 and NBS1 polymorphisms and acute skin toxicity for patients with normal weight and overweight (Table 1), which were, however, not statistically significant. When combined effects of alleles were examined, there was neither a significant trend (P trend = 0.08) for increased protection associated with increasing number of potentially protective alleles (XRCC3 Thr241Thr, XRCC2 Arg188His, and NBS1 Glu185Gln) in normal weight patients nor significant risk alterations (hazard ratio for carriers of three and more alleles, 0.26; 95% confidence interval, 0.04-1.66; data not shown).
Table 1. Genetic polymorphisms in DNA DSB repair genes XRCC3, XRCC2, and NBS1 and risk of developing acute skin toxicity after radiotherapy investigated in all participants and stratified by body mass index

<table>
<thead>
<tr>
<th>Gene polymorphism</th>
<th>Total population</th>
<th>Normal weight (BMI ≤ 25.0)</th>
<th>Overweight (BMI &gt; 25.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.* HR (95% CI) ¹</td>
<td>No.* HR (95% CI) ¹</td>
</tr>
<tr>
<td>XRCC3 Thr²⁴¹Met</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr/Thr</td>
<td>156/21</td>
<td>1.00</td>
<td>85/5</td>
</tr>
<tr>
<td>Thr/Met</td>
<td>212/41</td>
<td>1.34 (0.79-2.29)</td>
<td>104/13</td>
</tr>
<tr>
<td>Met/Met</td>
<td>76/14</td>
<td>1.28 (0.64-2.56)</td>
<td>38/4</td>
</tr>
<tr>
<td>Met carrier</td>
<td>288/55</td>
<td>1.33 (0.80-2.21)</td>
<td>142/17</td>
</tr>
<tr>
<td>XRCC2 Arg¹⁸⁸His</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>387/71</td>
<td>1.00</td>
<td>194/21</td>
</tr>
<tr>
<td>Arg/His</td>
<td>55/5</td>
<td>0.56 (0.22-1.39)</td>
<td>34/2</td>
</tr>
<tr>
<td>His/His</td>
<td>3/1</td>
<td>0.95 (0.13-7.02)</td>
<td>0/0</td>
</tr>
<tr>
<td>His carrier</td>
<td>58/6</td>
<td>0.60 (0.26-1.39)</td>
<td>34/2</td>
</tr>
<tr>
<td>NBS1 Gln¹⁸⁵Gln</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln/Glu</td>
<td>196/36</td>
<td>1.00</td>
<td>110/16</td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>210/35</td>
<td>0.94 (0.58-1.52)</td>
<td>104/7</td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>39/6</td>
<td>0.90 (0.37-2.22)</td>
<td>14/0</td>
</tr>
<tr>
<td>Gln carrier</td>
<td>249/41</td>
<td>0.93 (0.59-1.49)</td>
<td>118/7</td>
</tr>
</tbody>
</table>

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; BMI, body mass index; NC, not calculated.

¹Number of participants/patients with acute side effects.

¹Adjusted for BMI, hospital (four clinics), and photon beam quality for whole breast (two categories) and for boost irradiation (no boost and four categories; ref. 9).

¹ interaction = 0.41.

¹ interaction = 0.08.

Discussion

Our data did not support a significant association of risk of acute skin toxicity in breast cancer patients receiving radiotherapy after breast-conserving surgery with the XRCC3 Thr²⁴¹Met, XRCC2 Arg¹⁸⁸His, and NBS1 Gln¹⁸⁵Gln polymorphisms. The possibility of an interaction between genotypes and body mass index cannot be excluded because of the small number of participants in some of the genotype categories. Nevertheless, our study is one of the largest reported and has >80% power to detect a 1.9-fold risk in carriers of the XRCC3 Thr²⁴¹Met allele or the NBS1 Gln¹⁸⁵Gln allele in the overall study population (see also ref. 13). We minimized sources of bias in our study by restriction on tumor type and type of side effects and accounted for confounding by treatment-related or patient-related characteristics in the data analysis. Analysis for effect modification by body mass index was driven by the previous observation of differential associations between skin toxicity and DNA repair gene polymorphisms in the base excision repair genes XRCC1 and APE1 by body mass index (9, 13).

To our knowledge, this is the first study to investigate the association between XRCC2 Arg¹⁸⁸His and NBS1 Gln¹⁸⁵Gln polymorphisms and acute side effects of radiotherapy. For XRCC3 Thr²⁴¹Met, the Thr/Thr genotype was reported to be correlated with increased risk of late effects in irradiated breast cancer patients, such as s.c. fibrosis and telangiectasia (15). This finding was not confirmed in another study of late-adverse radiotherapy effects in gynecologic tumors (16). Our results weakly implicated the XRCC3 Thr²⁴¹Met allele as risk allele and may differ from the other studies because we focused on acute side effects rather than late effects of radiotherapy, which are not necessarily related (17).

All three polymorphisms investigated were predicted to have a maximal effect on cellular, and possibly clinical, function using an algorithm based on allele frequency, potential functional effect, and results from previous epidemiologic studies (18). The XRCC3 Thr²⁴¹Met allele has in fact been associated with an increased number of micronuclei in peripheral lymphocytes of humans exposed to ionizing radiation (5, 6). No differences in homology-directed repair of DSB have, however, been found between the wild-type and the variant XRCC3 protein (19), and DSB repair in vitro and acute normal tissue reaction after radiotherapy of breast cancer patients were not correlated (20). Thus, the polymorphisms investigated, although apparently functional, may not give total information about variability in gene function. In the future, a more detailed haplotype analysis of the genes and a comprehensive analysis, including variants in genes of both DSB repair pathways (homologous recombination and nonhomologous end joining), and consideration of late effects of radiotherapy will be necessary.

Acknowledgments

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References


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