Polymorphisms in the DNA Repair Gene XRCC1, Breast Cancer Risk, and Response to Radiotherapy

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Abstract
The study goal was to examine the association of three polymorphisms in the XRCC1 gene (Arg194Trp, Arg280His, and Arg399Gln) involved in repairing DNA damage produced by ionizing radiation, a known breast cancer (BC) risk factor, with BC incidence and the possibility of developing an adverse radiotherapy response. Genomic DNA from 254 BC cases, 70 of whom were adverse radiotherapy responders [radiation-sensitive breast cancer (RS-BC) patients], and 312 female blood donors were genotyped using either TaqMan technology or variant specific restriction enzyme digestion. Neither donors were genotyped using either TaqMan technology nor variant specific restriction enzyme digestion. Neither

Introduction
It is estimated that 5% of BC cases are related to rare but highly penetrant mutations in genes such as BRCA1 and BRCA2 (1, 2). The concept that in addition to such genes, there are other low penetrant genes predisposing to BC in a substantial proportion of cases is supported by epidemiological studies (3–6) and studies on the heritability of radiation sensitivity and defective DNA repair in families of patients with BC (7–10). At present, the identity of the putative genes is unknown, but the characteristics of the study population would suggest that some of them are involved in aspects of the normal cellular response to IR. In addition, mutants or variants in such genes must be relatively common within the normal population and present at high frequency among BC patients. A number of SNPs have been identified in genes involved in the repair of DNA damage produced by known or suspected risk factors for BC such as IR, heterocyclic amines, alcohol, estrogen, and diet (3, 11, 12).

XRCC1 is a DNA repair protein required for the efficient repair of DNA damage caused by IR, oxidative stress, and DNA methylating agents. The mouse XRCCI+/− gene knockout mutation is lethal, and mutations in XRCC1 result in an increased sensitivity to these agents and decreased genetic stability, including increased frequencies of spontaneously or induced chromosome translocations or deletions (Ref. 13 and the references therein). This protein has been implicated in every step in the repair of abasic sites through the short-patch branch of the BER through its action as both a scaffold and modulator of the different enzymes involved in BER (14).

XRCC1 interacts with DNA polymerase β, DNA ligase III, PARP-1, and APE1 (13, 14). Three domains have been identified within this 633-amino acid protein: the NH2-terminal domain (residues 1–183), which interacts with DNA polymerase β and DNA containing a single-strand break; the COOH-terminal BRCT-II (residues 358–633), which interacts with the COOH-terminal domain of DNA ligase III; and a central BRCT-I domain (residues 315–403), which interacts with PARP-1. Eight SNPs have been documented in XRCCI (12, 15), and six of these occur at residues that are conserved between the human and mouse. Two of the variants (Pro161Leu and Phe173Leu) occur in the NH2-terminal domain, and three (Arg194Trp, Arg280His, and Pro309Ser) occur in the linker region separating the NH2-terminal domain from the central BRCT-I domain. The Arg399Gln variant is located within the PARP-binding domain in BRCT-I, and the remaining two (Arg560Trp and Tyr576Ser) are located in the COOH-terminal domain. The 399Gln variant allele is the most frequently found.
it has been reported to be present at an allele frequency of around 0.35 in European Caucasians, whereas the 194Trp and 280His variant alleles are rarer polymorphisms (approximate allele frequencies of 0.08 and 0.04, respectively; Refs. 16–18). The allele frequencies of the other five variants are between 0.005 and 0.01 (15), and their impact on the XRCC1 protein has not been investigated to date at either a functional level or in cancer association studies.

The Arg399Gln polymorphism has been extensively investigated, and the presence of the variant allele has been shown to be associated with measurable reduced DNA repair capacity as assessed by the persistence of DNA aflatoxin B1 adducts (17) and DNA damage measured by 32P postlabeling of bulky DNA adducts (19–21), increased radiation sensitivity (22), increased RBC glycerophosph A mutations (17), elevated levels of sister chromatid exchanges (19, 23), and prolonged cell cycle delay (24). It has also been associated with an increased risk to fail 5-fluorouracil/oxaliplatin chemotherapy in advanced colorectal cancer (25). Only one study has examined the influence of the codon 194Trp allele on the function of the XRCC1 protein with no association being found with the Gp cell cycle delay seen in some BC patients (24). No such data exist for the codon 280 polymorphism.

A large number of epidemiological studies have assessed the association of the Arg399Gln polymorphism with cancer incidence (12, 16, 18, 19, 23, 26–39), with both increases and decreases in cancer frequencies being found depending on the tumor type and levels of environmental exposures. Three studies have examined the role of XRCC1 polymorphisms specifically in relation to BC risk. A significant association was found between the codon 399 Arg/Gln or Gln/Gln genotypes compared with Arg/Arg and risk among African-American women (OR, 1.7; 95% CI, 1.1–2.4) but not among white women (28). Similar results were reported by Smith et al. (38), with no significant differences in the variant allele frequencies at codon 399 being noted between Caucasian cases and controls; however, the 194Trp allele was weakly associated with BC risk (OR, 1.98; 95% CI, 0.85–4.63). Kim et al. (40), however, found that the codon 194 polymorphism had no influence on BC risk in Korean women, whereas homozygosity for the codon 399Gln allele increased the risk 2.4-fold (95% CI, 1.2–4.72). The risk increased to 3.8-fold (95% CI, 1.4–10.30) in premenopausal women. None of these studies examined the role of the codon 280 variant.

To clarify the possible association of these three XRCC1 variants with an increased BC risk and, in particular, the roles of the codon 194 and 280 polymorphisms, we have determined their frequency in BC patients compared with a group of age-matched blood donor controls. In addition, we have examined whether differences in allele frequencies were found between patients who either developed or did not develop adverse reactions to radiotherapy. It is well known from clinical observations that a considerable interpatient heterogeneity in the normal tissue reactions can be seen in cancer patients after radiotherapy, varying from very mild to severe and occasionally lethal. We hypothesized that the less common alleles would be associated with an increased risk of BC and with this radiation sensitivity seen in some patients.

Materials and Methods

Subjects. Local ethical committees approved the study design. The BC cases were recruited over the period of February 1996 to April 2002 from among women treated in the Radiotherapy Department at Lyon-Sud Hospital (Pierre-Bénite, France). The average treatment dose was 50 Gy, in dose fractions of 2.5 Gy over a 6-week period, followed by a 10-Gy boost to the tumor bed (41). A total of 254 BC cases over the age of 35 years were enrolled (average age at radiotherapy, 56.8 ± 10.2 years; range, 35–81 years). Seventy BC cases (average age at radiotherapy, 58.8 ± 10.8 years; range, 35–81 years) who displayed adverse reactions to their radiotherapy as classified and graded by the European Organization for Research and Treatment of Cancer (42), in the 2-year period after the start of their radiotherapy formed the RS-BC group. This group included BC cases who showed early, early and late, or late normal tissue reactions. None of the remaining 184 BC cases (average age at radiotherapy, 56.1 ± 9.9 years; range, 35–78 years) developed any adverse reactions to their radiotherapy over a period of 2 years after the start of their treatment (the NRS-BC group). For all cases, a blood sample was collected after written informed consent had been obtained, on the occasion of a routine control visit to the radiotherapy clinic.

Three hundred and twelve control blood samples were obtained from female blood donors over the age of 35 years living in the catchment area of the hospital through community-based collections of the Regional Blood Transfusion Service (average age at sample collection, 54.5 ± 6.7 years; range, 35–66 years). The health status of these controls remained unknown, although it should be noted that any bias from such an individual having cancer would be expected to favor a null result.

Laboratory Methods. DNA was extracted from either whole blood or lymphoblastoid cells for the BC patients and from the Buffy coat for the controls using Qiagen (Qiagen SA, Courtaboeuf, France) genomic extraction kits. DNA concentrations were determined using the Pico-green double-stranded DNA quantitation kit (Molecular Probes, Eugene, OR).

The XRCC1 Arg399Gln polymorphism, a G→A transition in exon 10, was genotyped by restriction enzyme digestion of a PCR fragment with the MspI enzyme (New England BioLabs, Beverly, MA); the Arg allele contains the MspI site, whereas the Gln allele is not digested by this restriction enzyme. The PCR primers (F, sense or forward; R, antisense or reverse) are as follows: XRCC1-exon10-F, CTAACCTggCATCTTCACTTg; and XRCC1-exon10-R, CATTggCCAgCACAgATAAg.

The PCR reaction was carried out in a total reaction volume of 25 μl containing 50 ng of genomic DNA, 1× Taq platinum buffer, 2 mM MgCl2, 100 μM deoxynucleotide triphosphates, 0.4 μM each primer, and 2.5 units of Platinum® Taq polymerase (In Vitrogen SARL, Cergy Pontoise, France). The cycling conditions were 5 min at 94°C; followed by 42 cycles of 94°C for 30 s, 61°C for 30 s, and 72°C for 30 s; with a final extension at 72°C for 5 min. Ten μl of the 198-bp PCR product was digested overnight with 20 units of MspI. The digestions were performed according to the manufacturer’s instructions, and the fragments were analyzed by gel electrophoresis on a 3% agarose gel. The homozygous AA allele produced three fragments of 198, 145, and 53 bp, and the heterozygous G/A allele produced a single 198-bp product, the homozygous G/G allele produces 145- and 53-bp fragments, and the heterozygous G/A allele produced two fragments of 198, 145, and 53 bp. DNA samples known to be carrying the variant allele were included in each analysis to ensure accurate genotyping, and two investigators read all gels.

The polymorphisms at codons 194 (a C→T substitution in exon 6) and at codon 280 (an A→G in exon 9 of XRCC1) were genotyped using the TaqMan assay (Applied Biosystems, Courtaboeuf, France). PCR primers and probes were designed using Primer Express software (Applied Biosystems). The
primers and allele-specific probes with their corresponding
primer, 2.5 μM wild-type probe, 2.5 μM variant probe, and 20 ng of genomic DNA in a total volume of 11 μL. The PCR was run on an Applied Biosystems GenAmp 9700 thermocycler under the following conditions: 95 °C for 10 min; followed by 45 cycles of 94 °C for 30 s and 62 °C for 1 min. The genotype characterization was made by comparison of the profiles of samples being tested with those of wild-type and variant samples included on each plate. In addition, at least 30 samples were sequenced for each polymorphism (exon 6, 9, and 10), and the results were identical to that of the initial analysis (restriction enzyme or TaqMan; data not shown).

The reaction mixture for these two polymorphisms was as follows: 1× TaqMan Universal PCR Master Mix, 1 μM each primer, 2.5 μM wild-type probe, 2.5 μM variant probe, and 20 ng of genomic DNA in a total volume of 11 μL. The PCR was run on an Applied Biosystems GenAmp 9700 thermocycler under the following conditions: 95 °C for 10 min; followed by 45 cycles of 94 °C for 30 s and 62 °C for 1 min. The genotype characterization was made by comparison of the profiles of samples being tested with those of wild-type and variant samples included on each plate. In addition, at least 30 samples were sequenced for each polymorphism (exon 6, 9, and 10), and the results were identical to that of the initial analysis (restriction enzyme or TaqMan; data not shown).

Statistical Methods. Departures from Hardy-Weinberg equilibrium were assessed by comparing the observed and expected genotype frequencies. Statistical significance was evaluated using χ² tests. Haplotype frequencies were reconstructed using the Arlequin software and the Genotype Transposer (43).

In determining the risk of BC in general, all cases were compared with controls. In determining the risk of radiosensitivity, NRS-BC cases were used as the control group. ORs based on allele or haplotype frequencies were determined using Fisher’s exact test, with the wild-type alleles being used as the reference group.

For genotypic data, logistic regression analysis was used, stratifying cases and controls by the following age groups: <45 years; 45–54 years; 55–64 years; 65–74 years; >74 years. The age at radiation therapy was used for the cancer cases. All analysis was performed using Stata 7.0. All statistical tests are two-sided.

Results
The allele frequencies for the three polymorphisms in the controls are very similar to those reported previously in Caucasian control subjects from America and Europe. The codon 399Gln allele was present at a frequency of 0.36, the codon 194Trp allele was present at a frequency of 0.07, and the codon 280His allele was present at a frequency of 0.05. All of the genotype distributions were in Hardy-Weinberg equilibrium in both the cases and the controls. The associations between the three XRCCI polymorphisms and BC risk are given in Table 1.

A positive association was found between the exon 9 codon 280His allele and BC (BC versus controls: OR, 1.80; 95% CI, 1.07–3.05). This association was seen both in the cases and the controls. The associations between the three XRCCI polymorphisms and BC risk are given in Table 1.

The exon 6 194Trp allele was found more frequently in the controls: OR, 0.95; 95% CI, 0.74–1.23. This association was seen both in the cases and the controls. The associations between the three XRCCI polymorphisms and BC risk are given in Table 1.

Table 1  Association between XRCCI polymorphisms and BC and risk of adverse reaction to radiotherapy

<table>
<thead>
<tr>
<th></th>
<th>BC cases (N = 254)</th>
<th>Controls (N = 312)</th>
<th>OR (95% CI)</th>
<th>RS-BC (N = 70)</th>
<th>NRS-BC (N = 184)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exon 6 codon</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>194 Arg</td>
<td>472</td>
<td>581</td>
<td>1.00</td>
<td>125</td>
<td>347</td>
<td>1.00</td>
</tr>
<tr>
<td>Trp</td>
<td>36</td>
<td>43</td>
<td>1.03 (0.62–1.67)</td>
<td>15</td>
<td>21</td>
<td>1.98 (0.92–4.17)</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>219</td>
<td>270</td>
<td>1.00</td>
<td>56</td>
<td>163</td>
<td>1.00</td>
</tr>
<tr>
<td>Arg/Thr</td>
<td>34</td>
<td>41</td>
<td>0.95 (0.56–1.61)</td>
<td>13</td>
<td>21</td>
<td>1.81 (0.84–3.96)</td>
</tr>
<tr>
<td>Thr/Thr</td>
<td>1</td>
<td>1</td>
<td>1.61 (0.10–26.10)</td>
<td>1</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td><strong>Exon 9 codon</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>280 Arg</td>
<td>467</td>
<td>595</td>
<td>1.00</td>
<td>129</td>
<td>338</td>
<td>1.00</td>
</tr>
<tr>
<td>His</td>
<td>41</td>
<td>29</td>
<td>1.8 (1.07–3.05)</td>
<td>11</td>
<td>30</td>
<td>0.96 (0.42–2.04)</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>214</td>
<td>283</td>
<td>1.00</td>
<td>60</td>
<td>154</td>
<td>1.00</td>
</tr>
<tr>
<td>Arg/Thr</td>
<td>39</td>
<td>29</td>
<td>1.80 (1.04–3.08)</td>
<td>9</td>
<td>30</td>
<td>0.76 (0.33–1.73)</td>
</tr>
<tr>
<td>Thr/Thr</td>
<td>1</td>
<td>0</td>
<td>NC</td>
<td>1</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td><strong>Exon 10 codon</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>399 Arg</td>
<td>331</td>
<td>400</td>
<td>1.00</td>
<td>85</td>
<td>246</td>
<td>1.00</td>
</tr>
<tr>
<td>Gln</td>
<td>177</td>
<td>224</td>
<td>0.95 (0.74–1.23)</td>
<td>55</td>
<td>122</td>
<td>1.30 (0.85–1.99)</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>109</td>
<td>127</td>
<td>1.00</td>
<td>24</td>
<td>85</td>
<td>1.00</td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>113</td>
<td>146</td>
<td>0.96 (0.66–1.40)</td>
<td>37</td>
<td>76</td>
<td>1.80 (0.97–3.31)</td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>32</td>
<td>39</td>
<td>1.00 (0.57–1.76)</td>
<td>9</td>
<td>23</td>
<td>1.43 (0.57–3.54)</td>
</tr>
</tbody>
</table>

* Used as reference category.
* CI were calculated using Fisher’s exact test.
* Logistic regression, corrected for age.
* NC, not calculated.

Fisher’s exact test, with the wild-type alleles being used as the reference group.

For genotypic data, logistic regression analysis was used, stratifying cases and controls by the following age groups: <45 years; 45–54 years; 55–64 years; 65–74 years; >74 years. The age at radiation therapy was used for the cancer cases. All analysis was performed using Stata 7.0. All statistical tests are two-sided.

Results
The allele frequencies for the three polymorphisms in the controls are very similar to those reported previously in Caucasian control subjects from America and Europe. The codon 399Gln allele was present at a frequency of 0.36, the codon 194Trp allele was present at a frequency of 0.07, and the codon 280His allele was present at a frequency of 0.05. All of the genotype distributions were in Hardy-Weinberg equilibrium in both the cases and the controls. The associations between the three XRCCI polymorphisms and BC risk are given in Table 1.

A positive association was found between the exon 9 codon 280His allele and BC (BC versus controls: OR, 1.80; 95% CI, 1.07–3.05). This association was seen both in the RS-BC and NRS-BC cases (RS-BC versus NRS-BC: OR, 0.96; 95% CI, 0.42–2.04), indicating that the association was with BC status and not the radiation sensitivity seen in some of the patients. No association was found with BC and either the exon 6 194Trp allele or the exon 10 codon 399 polymorphism (BC versus controls: OR, 0.95; 95% CI, 0.74–1.23).

The exon 6 194Trp allele was found more frequently in the RS-BC cases (10.7%) compared with the controls (6.9%) and the NRS-BC cases (10.7%) or the exon 9 codon 399 polymorphism (BC versus controls: OR, 1.80; 95% CI, 1.07–3.05). This association was seen both in the cases and the controls. The associations between the three XRCCI polymorphisms and BC risk are given in Table 1.
radiotherapy. The frequency of the exon 10 variant allele was the same in the RS-BC and NRS-BC cases (RS-BC versus NRS-BC; OR, 1.3; 95% CI, 0.85–1.99).

The reconstructed haplotypes are shown in Table 2. It should be noted that the rare alleles in exon 9 and exon 10 are never seen on the same haplotype. One haplotype Trp, Arg, Gln (exon 6, exon 9, and exon 10, respectively) was seen in only four RS-BC cases. The intragenic association of the three polymorphisms is shown in Table 3. The reference group was defined as those individuals with the homozygote wild-type alleles at all three SNPs and used to calculate the BC risks associated with the different genotype combinations. Two genotype combinations were only seen in BC cases (both were in 1.47) 4 15 0.96 (0.27–2.04).

The codon 280His allele was present in four combinations in the individuals tested. One of these, in which the codon 194Trp allele was found more frequently in the RS-BC patients (7 of 184; OR, 4.33; 95% CI, 1.24–15.12).

Discussion

This study has investigated possible associations between the presence of three variant XRCC1 alleles and BC risk and the risk of developing an adverse reaction to radiotherapy treatment. The analysis of the individual SNPs showed that only the 280His allele was associated with an increased risk of BC. However, the analysis of the different combinations of the variants seen in each individual showed that this allele, in combination with the Gln variant allele at codon 399, was found more frequently in the BC cases than in the controls. The 399Gln allele alone did not appear to be associated with an increased BC risk from the analysis of the individual SNPs. One limitation of this study is that demographic information on the study population is not available, and in particular, no detailed information on study population ethnicity was available. Secondly, the controls were from a different source, although they were living in the catchment area of the hospital. However, the fact that the genotype distributions of cases and controls were in Hardy-Weinberg equilibrium suggests that the allele-specific associations observed were not due to selection bias. The results obtained are in agreement with the findings of Arg280/Gln399 on the second chromosome. The assessment of the intragenic association of the three polymorphisms also showed that the codon 399Gln allele in combination with the homozygote wild-type allele at codon 280 and the codon 194Trp allele was found more frequently in the RS-BC patients than in the NRS-BC cases (7 of 70 versus 7 of 184; OR, 4.33; 95% CI, 1.24–15.12).

### Table 2 Reconstructed XRCC1 haplotypes

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>BC cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>RS-BC</th>
<th>NRS-BC</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>258</td>
<td>328</td>
<td>1.00 (^{bc} )</td>
<td>63</td>
<td>195</td>
<td>1.00 (^{bc} )</td>
</tr>
<tr>
<td>Arg/His</td>
<td>41</td>
<td>29</td>
<td>1.80 (1.06–3.08)</td>
<td>30</td>
<td>15</td>
<td>1.13 (0.48–2.49)</td>
</tr>
<tr>
<td>Arg/Trp</td>
<td>173</td>
<td>224</td>
<td>0.98 (0.75–1.28)</td>
<td>51</td>
<td>122</td>
<td>1.29 (0.82–2.04)</td>
</tr>
<tr>
<td>Trp/Arg</td>
<td>32</td>
<td>43</td>
<td>0.95 (0.56–1.53)</td>
<td>11</td>
<td>21</td>
<td>1.62 (0.67–3.74)</td>
</tr>
<tr>
<td>Trp/Trp</td>
<td>4</td>
<td>0</td>
<td>0.83-- (^{\infty} )</td>
<td>4</td>
<td>0</td>
<td>1.97-- (^{\infty} )</td>
</tr>
</tbody>
</table>

*The haplotype is defined as the allele present on exon 6, exon 9, and exon 10, respectively.

### Table 3 Intragenic association of the XRCC1 polymorphisms

<table>
<thead>
<tr>
<th>Exon 6, codon 194</th>
<th>Exon 9, codon 280</th>
<th>Exon 10, codon 399</th>
<th>BC cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>RS-BC</th>
<th>NRS-BC</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>Arg/Arg</td>
<td>Arg/Arg</td>
<td>70</td>
<td>82</td>
<td>1.00</td>
<td>14</td>
<td>56</td>
<td>1.00</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>Arg/Arg</td>
<td>Arg/Gln</td>
<td>81</td>
<td>122</td>
<td>0.82 (0.52–1.29)</td>
<td>25</td>
<td>56</td>
<td>1.82 (0.84–3.92)</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>Arg/Arg</td>
<td>Gln/Gln</td>
<td>30</td>
<td>39</td>
<td>0.91 (0.49–1.67)</td>
<td>7</td>
<td>23</td>
<td>1.21 (0.43–3.6)</td>
</tr>
<tr>
<td>Arg/Trp</td>
<td>Arg/Arg</td>
<td>Arg/Arg</td>
<td>16</td>
<td>24</td>
<td>0.68 (0.31–1.47)</td>
<td>4</td>
<td>12</td>
<td>1.27 (0.34–4.66)</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>Arg/His</td>
<td>Arg/Gln</td>
<td>18</td>
<td>9</td>
<td>2.54 (1.04–6.22)</td>
<td>5</td>
<td>13</td>
<td>1.61 (0.48–5.45)</td>
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<tr>
<td>Arg/Arg</td>
<td>Arg/His</td>
<td>Arg/Arg</td>
<td>19</td>
<td>18</td>
<td>1.16 (0.54–2.50)</td>
<td>4</td>
<td>15</td>
<td>0.96 (0.27–3.45)</td>
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<td>Arg/Arg</td>
<td>Arg/Trp</td>
<td>Arg/Arg</td>
<td>2</td>
<td>2</td>
<td>1.51 (0.2–1.12)</td>
<td>0</td>
<td>2</td>
<td>NC</td>
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<tr>
<td>Arg/Trp</td>
<td>Arg/Arg</td>
<td>Arg/Gln</td>
<td>14</td>
<td>15</td>
<td>1.05 (0.44–2.51)</td>
<td>7</td>
<td>7</td>
<td>4.33 (1.24–15.12)</td>
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<tr>
<td>Trp/Trp</td>
<td>Arg/Arg</td>
<td>Arg/Arg</td>
<td>1</td>
<td>1</td>
<td>1.55 (0.09–25.45)</td>
<td>1</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td>Trp/Trp</td>
<td>Arg/Arg</td>
<td>Gln/Gln</td>
<td>2</td>
<td>0</td>
<td>NC-- (^{d} )</td>
<td>1</td>
<td>0</td>
<td>NC</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>His/His</td>
<td>Arg/Arg</td>
<td>1</td>
<td>0</td>
<td>NC-- (^{d} )</td>
<td>1</td>
<td>0</td>
<td>NC</td>
</tr>
</tbody>
</table>

*The reference group was defined as those individuals carrying the wild-type allele in the homozygote state for all three SNPs.

*The alleles present at each position.

*Adjusted for age.

*NC, not calculated.
Duell et al. (28) and Smith et al. (38). Neither group reported an association of the codon 399 variant allele alone with an increased BC risk in white American women or Caucasian women. The study of Smith et al. (38) noted a weak association of the 194Trp allele and BC risk and, interestingly, also found a potential gene-gene interaction between this variant and the XRCC3 241Met allele and BC risk (adjusted OR, 8.74; 95% CI, 1.13–67.53). Kim et al. (40), however, found that the 194 variant allele had no influence on BC risk in Korean women. They did note that an increased risk was associated with the number of 399Gln alleles carried, with homozygosity for the variant allele increasing the BC risk by 2.4 fold. A possible association between the presence of the 280His allele and BC has not been examined previously.

The exon 6 codon 194Trp variant allele was the only one studied that showed any difference in allele frequency between the RS-BC and the NRS-BC cases, although this difference did not quite reach statistical significance. The intragenic association of this variant with the codon 399Gln allele was found more frequently in the RS-BC cases than the NRS-BC cases (OR, 4.33; 95% CI, 1.24–15.12), suggesting a possible interaction between the presence of these two variants on developing an adverse reaction to radiotherapy. The severity of tissue response to radiotherapy varies considerably between individuals, with small proportions of patients receiving radiotherapy developing long-term radiation damage. It is thought that whereas some of these differences are due to factors related to the treatment (fractionation schedule, volume of treatment field, and so forth) and other predisposing factors such as surgery, chemotherapy, or diabetes, they cannot fully explain patient-to-patient differences. Studies correlating a variety of end points such as in vitro radiosensitivity of either fibroblasts or lymphocytes or micronucleus induction with the risk of developing radiation reactions have lead to contradictory results and conclusions (see for instance, Refs. 44 and 45 and the references therein). Part of this variation is probably due to genetically determined intrinsic differences in the cellular response to radiation (46). Besides the known radiosensitivity syndromes such as ataxia telangiectasia and Nijmegen breakage syndrome (47) and the finding of gene defects in DNA ligase IV (48), Fanconi anemia (49) and hHR21 (50) in radiosensitive cancer patients, the underlying causes of this radiosensitivity are poorly understood. To date, no genetic factors that might specifically influence the temporal occurrence of these adverse reactions have been identified, nor is there evidence to suggest that the genetic determinants of early or late reactions are different. The small numbers of BC cases showing either an early adverse reaction, a late reaction, or both in our study exclude the possibility of addressing whether the XRCCI variants can influence the temporal aspect of this radiosensitivity. Additional association studies in well-characterized large cohorts analyzing genes involved in the different mechanisms of the cellular response to radiation, such as DNA damage detection and repair or free radical detoxification, could prove a promising approach to identify genes that influence the different aspects of this adverse response to radiotherapy.

The mechanistic basis for the present findings remains unclear. It has been suggested that the Arg280His polymorphism may result in lower DNA repair ability compared with homozygous wild type (35). However, there are no studies examining the potential impact of this polymorphism on XRCCI function, either alone or in combination with the codon 399Gln allele, in the BER pathway or its interaction with DNA polymerase β, DNA ligase III, PARP-1, and APE1. Similarly, there are no published studies examining the impact of the exon 6 codon 194Trp on XRCCI function. Both the codon 194Trp and 280His variants are located in a linker region (residues 158–310) between the NH2-terminal domain and the central BRCT domain, BRCT-I, which consists of polar Pro, Ser, and Arg/Lys-rich regions. Within this segment, there is a region (residues 158–250, conserved region 1) that is highly conserved between the human, mouse, and hamster proteins (>80% sequence identity). These residues have significant hydrophobicity and have been predicted to have a secondary structure suggestive of folding to form an inner domain. A possible role for this linker region, which includes the predicted inner domain, might be facilitation of DNA binding because this segment has a high pI (10.7) and overall positive charge (51).

The Arg399Gln is located within the central BRCT-I domain that has been shown to bind the BRCT domain of PARP (52). The presence of the 399Gln variant has been correlated with persistence of DNA damage (17, 19–21), mutation induction (17), IR-induced cell cycle delay (24), increased radiation sensitivity (22), and elevated formation of SCEs (19, 23). A recent functional study has compared the ability of the codon 399Arg and 399Gln alleles to conduct single-strand break repair and mediate cell survival in an isogenic background. The two alleles were equally able to complement both the single-strand break repair defect and sensitivity to methyl methane sulfonate in XRCCI-deficient Chinese hamster EM9 cells. Given that the BRCT-I domain, in which this polymorphism is located, is critical for these functions, these experiments would suggest that the presence of the codon 399Gln allele has little effect on the BRCT-I domain or XRCCI function, at least under the experimental conditions used (53). It will thus be important to carry out similar experiments in human cells with XRCCI variant proteins expressing the codon 280His allele alone and in combination with the codon 399Gln allele to establish if there is a functional basis for the increased BC risk seen in this study. A larger study will be necessary to validate our data that suggest that the XRCCI codon 194 and 399 genotype may influence the response of BC patients to radiotherapy, together with functional studies to establish its mechanistic basis.

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