Abstract. Background: Single nucleotide polymorphisms (SNPs) in a DNA-repair gene, X-Ray repair cross complementing group 1 (XRCC1), have been associated with the survival of patients with breast cancer. We investigated the predictive value of XRCC1 SNP (rs25487) in patients with early breast cancer. Patients and Methods: The XRCC1 rs25487 genotypes of 411 Finnish patients with breast cancer were analyzed by a polymerase chain reaction-restriction fragment length polymorphism-based method. Survival was assessed by Kaplan–Meier method and Cox regression analysis according to the XRCC1 genotypes in specified adjuvant treatment groups. Results: The rs25487 variant AA genotype was associated with worse breast cancer-specific and overall survival in 238 patients receiving postoperative radiotherapy (p=0.031 and p=0.030, respectively). The AA genotype predicted worse breast cancer-specific survival among 75 patients treated with adjuvant chemotherapy (p=0.047). Conclusion: The XRCC1 rs25487 genotype may predict the outcome of postoperative radiotherapy and adjuvant chemotherapy in breast cancer.

Approximately 1.4 million women worldwide are diagnosed annually with breast cancer (1). This malignancy is also the most frequent cause of cancer mortality among females with 460,000 deaths every year (1). Adjuvant treatments, including hormonal treatment, chemotherapy, and radiotherapy, have been shown to improve the long-term survival of patients with breast cancer (2-4). Postoperative radiotherapy improves the 15-year breast cancer-specific survival (BCSS) by an absolute risk reduction of 3.8% (2), the benefits being similar in patients treated with breast-conserving surgery and patients treated with mastectomy (5). It also reduces the 10-year risk of any recurrence with an absolute reduction of 15.7% (2). However, radiation therapy of breast cancer is associated with a risk of both acute and late adverse effects including breast oedema, pulmonary and cardiac complications, and radiation-induced malignancies (6, 7).

Radiation therapy and cytotoxic treatment destroy cancer cells by inducing DNA damage. Therefore, the outcome of these treatments may be dependent on the efficacy of DNA repair systems. The X-Ray repair cross complementing group 1 (XRCC1) protein is essential involved both in the repair of single-strand breaks and in base excision repair. Instead of being enzymatically active, the XRCC1 protein interacts with multiple enzymes that act on SSB, including polymerase β (POLβ), poly(ADP-ribose)polymerase, and ligase III (8).

One of the known single nucleotide polymorphisms (SNPs) of the XRCC1 gene is a G to A nucleotide substitution in codon 399 (rs25487) resulting in an amino acid change from arginine (Arg) to glutamine (Gln). Research on the functional effects of the $\text{Arg}^{399}\text{Gln}$ change has suggested that the variant AA genotype is associated with a 3- to 4-fold reduced DNA repair capacity (9). In addition, carriage of the variant A allele has been associated with an increase of chromosomal deletions (10).

Several studies have been conducted on the XRCC1 rs25487 polymorphism and its influence on the survival of patients with cancer, with controversial results (11-17). For example, the variant AA genotype has been associated with...
improved progression-free survival (PFS), BCSS, and overall survival (OS) among chemotherapy-treated patients with primary invasive breast cancer (14), whereas the rs25487 wild-type allele-containing genotypes (GG and AG) conferred a reduced risk of recurrence or death among patients with metastatic breast cancer receiving chemotherapy (12).

Studies on patients with lung or prostate cancer have provided evidence that the rs25487 polymorphism may be associated with the clinical efficacy of radiotherapy (11, 15). An impact of the rs25487 genotype has also been observed on response and survival in patients with bladder, esophageal, or rectal cancer treated with neoadjuvant chemoradiotherapy (CRT) (13, 16, 18).

The aim of this study was to determine the role of the XRCC1 rs25487 SNP as a predictor of the outcome for adjuvant therapies of breast cancer. To our knowledge, there are no previous studies investigating the influence of rs25487 polymorphism in the clinical outcome of patients with breast cancer receiving postoperative radiotherapy.

Patients and Methods

Study participants. This study is part of the Kuopio Breast Cancer Project, a prospective case–control study conducted at the Kuopio University Hospital. Altogether, 520 patients diagnosed with breast cancer were recruited during 1990-1995. Patients gave their written informed consent for the study. All patients were ethnic Finns. The study was approved by the ethical board of Kuopio University Hospital (approval date: 14th of December 1989, approval number: 112/95).

Information regarding clinical and pathological features, as well as the data on survival, was collected from the hospital registries. Only patients with primary local invasive breast cancer, known nodal status, and known rs25487 genotype were included in these analyses (n=411).

Altogether 241 patients with known rs25487 genotype were treated with postoperative radiotherapy. One patient was excluded from further analyses due to low total radiation dose (28 Gy), one patient received radiation to the nodal areas only, and one patient received only interstitial radiotherapy. Of the remaining 238 patients, 87 underwent lumpectomy and 151 were treated with mastectomy. Irradiation of the whole breast was given to 65 patients, whereas 173 patients were treated with radiotherapy both to the breast and the regional lymph nodes. Radiotherapy was delivered from a linear accelerator by photon/electron beams. The standard dose was a total of 50 Gy (range 44-56 Gy) given in 2-Gy daily fractions, five fractions per week. Four patients were given additional external booster (median dose 10 Gy, range 6-10 Gy), and 25 patients received interstitial radiotherapy in addition to the standard external radiotherapy (median dose 9 Gy, range 9-20 Gy). Seventy patients were additionally treated with adjuvant chemotherapy, 76 patients received adjuvant hormonal treatment, and four patients were treated with both chemotherapy and hormonal treatment.

Adjuvant chemotherapy was the only medical treatment for 75 patients. Five of them did not receive postoperative radiotherapy. Six patients were treated with CNF (500 mg/m² cyclophosphamide, 10 mg/m² mitoxantrone and 500 mg/m² 5-fluorouracil), whereas 69 patients received CMF (500 mg/m² cyclophosphamide, 40 mg/m² methotrexate, 500 mg/m² 5-fluorouracil). The median number of cycles was 6 (range 2-6).

Genotyping. A previously described polymerase chain reaction-restriction fragment length polymorphism-based method was used for the determination of the XRCC1 rs25487 genotypes (19). Two positive controls with known genotype and two negative controls were used within each PCR amplification batch, and two independent researchers interpreted the gel images to ensure the validity of genotyping.

Statistical analysis. The statistical analyses were performed using SPSS version 19.0. (IBM SPSS Statistics for Windows, Version 19.0., Armonk, NY, USA). Relapse-free survival (RFS) was assessed as the time from diagnosis to the first relapse, either local or distant. BCSS was defined as the time between diagnosis and death caused by breast cancer, whereas OS was measured as the time between diagnosis and death from any cause or the date of cutoff in February 2011. The univariate estimates of the survival were calculated by the Kaplan–Meier function. The hazard ratios (HRs) and 95% confidence intervals (CIs) were assessed by Cox proportional hazards model. Both the dominant and recessive models of the rs25487 were tested for the survival analyses. A p-value of 0.05 or less was considered to be statistically significant.

Results

Patients’ characteristics. Data on clinical characteristics and rs25487 genotypes of all the 411 patients are presented in Table I. The rs25487 genotypic frequencies in different treatment groups were similar to those described previously in Caucasian populations (8) and they were in Hardy–Weinberg equilibrium in all the treatment groups analysed.

XRCC1 rs25487 genotype is associated with the survival of patients treated with postoperative radiotherapy. The Kaplan–Meier survival functions showed a borderline significant difference in BCSS according to the rs25487 genotype in 238 patients receiving radical postoperative radiotherapy, the survival being worse for the patients carrying the homozygous rs25487 variant AA genotype (log-rank p=0.056). After adjusting for age, stage, chemotherapy, and hormonal treatment, the Cox regression survival analysis showed an increased risk for breast cancer death in patients homozygous for the variant A allele (p=0.031) (Table II, Figure 1b). Similarly, carriage of the AA genotype was associated with worse OS (p=0.030) (Table II, Figure 1c).

Association of XRCC1 rs25487 and survival of chemotherapy-treated patients. The Kaplan–Meier survival analysis did not reveal any significant overall differences in RFS, BCSS, or OS (log-rank p=0.50, p=0.13, and p=0.16, respectively). However, the multivariate analysis showed worse BCSS (p=0.047) for the patients with the rs25487 AA genotype compared with the patients carrying the wild-type G allele (Table II, Figure 1d).
The Kaplan–Meier analysis of all eligible patients showed worse BCSS for patients with the homozygous rs25487 variant AA genotype (log-rank $p=0.032$). The RFS and OS did not differ significantly in the univariate analysis when using the dominant model (log-rank $p=0.74$ and $p=0.17$, respectively).

After adjustment for age, stage, radiotherapy, chemotherapy, and hormonal treatment, the Cox regression analysis indicated that the patients with the variant AA genotype had worse BCSS ($p=0.014$) (Table II, Figure 1a) and a trend towards decreased RFS ($p=0.054$) compared with the patients with other genotypes (Table II).

**XRCC1 rs25487 genotype as a prognostic factor.** The Kaplan–Meier analysis of all eligible patients showed worse BCSS for patients with the homozygous rs25487 variant AA genotype (log-rank $p=0.032$). The RFS and OS did not differ significantly in the univariate analysis when using the dominant model (log-rank $p=0.74$ and $p=0.17$, respectively).
The univariate and multivariate analyses did not show any statistically significant differences in survival according to the rs25487 genotype in the cohort of patients who did not receive any type of adjuvant treatment, nor among patients receiving hormonal treatment (data not shown). Neither were there significant survival differences in the recessive models of any of the specified adjuvant treatment groups.

**Discussion**

In the present study, we investigated the impact of the XRCC1 rs25487 polymorphism on the survival of patients with breast cancer treated with postoperative radiotherapy, adjuvant chemotherapy, or hormonal treatment. Our results suggest that the homozygous rs25487 variant AA genotype may
predict inferior survival in patients treated with postoperative radiotherapy. In addition to this novel finding, we noticed an increased risk of death from breast cancer among chemotherapy-treated patients carrying the AA genotype. The importance of the role of XRCC1 rs25487 in cancer treatment efficacy and patient outcome is, however, still indistinct. rs25487 AA has been shown to be associated with worse survival after DNA-damaging adjuvant treatments. In line with this, the AA genotype has been reported to be associated with resistance to cytotoxic drugs in vitro (21). These results are contradictory to the laboratory experiments suggesting that the variant genotype results in less efficient repair of DNA damage induced by various agents including chemicals, light, and irradiation (9, 10). It is probable that inconsistent results are due to the variability in the study designs, cell lines, and methods used to measure DNA repair.

The level of XRCC1 protein expression has been shown to influence the outcome of radiotherapy. Sak et al. observed that a high level of XRCC1 expression was associated with improved cancer-specific survival in 90 patients with bladder cancer following radical radiotherapy (22). This finding was opposite to their working hypothesis that high expression of DNA repair enzyme would lead to resistance to radiotherapy and worse survival. As a potential explanation, they suggested that the repair of non-lethal base damage by base excision repair might increase the number of lethal double-strand breaks.

There is also controversy over results of the correlation between XRCC1 polymorphisms and XRCC1 protein expression. In an evaluation of XRCC1 expression of lymphoblastoid cells, the homozygous rs25487 AA variant genotype was associated with increased XRCC1 gene expression compared to the homozygous wild-type genotype (23). There was also an increase in the median nicking activity of a uracil substrate in the cell lines carrying the variant A allele, but the difference was not statistically significant (23). In contrast, in a study investigating breast cancer tissue samples from 39 patients, no association between the XRCC1 genotype and protein expression was noticed (24).

Our results are supported by a study including 95 patients with metastatic breast cancer, where the homozygous variant AA genotype was associated with improved survival in 58 patients receiving adjuvant chemotherapy, and the significant association persisted in the survival analysis of 98 patients receiving both adjuvant chemotherapy and radiotherapy (14). Similarly, patients treated with adjuvant chemotherapy combined with radiotherapy (n=335) were shown to have an OS advantage when carrying the variant AA genotype (26). In addition, in a study with patients with breast cancer receiving adjuvant

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### Table II. X-Ray repair cross complementing group 1 (XRCC1) rs25487 genotype and survival of patients with breast cancer.

<table>
<thead>
<tr>
<th>XRCC1</th>
<th>n</th>
<th>RFS (HR 95% CI)</th>
<th>p-Valuea</th>
<th>BCSS (HR 95% CI)</th>
<th>p-Valuea</th>
<th>OS (HR 95% CI)</th>
<th>p-Valuea</th>
<th>Median OS (years)</th>
</tr>
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<tr>
<td>All invasive cases</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>GG or AG</td>
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<td>1.00b</td>
<td>0.054</td>
<td>1.00b</td>
<td>0.014</td>
<td>1.00b</td>
<td>0.30</td>
<td>12.0</td>
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<td>AA</td>
<td>42</td>
<td>1.61 (0.99-2.60)</td>
<td></td>
<td>1.95 (1.15-3.32)</td>
<td>0.041</td>
<td>1.24 (0.82-1.87)</td>
<td>0.030</td>
<td>10.7</td>
</tr>
<tr>
<td>RT w/wo CT w/wo HT</td>
<td>238</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG or AG</td>
<td>214</td>
<td>1.00c</td>
<td>0.21</td>
<td>2.03 (1.07-3.85)</td>
<td>0.031</td>
<td>1.85 (1.06-3.24)</td>
<td>0.030</td>
<td>13.1</td>
</tr>
<tr>
<td>AA</td>
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<td>1.50 (0.80-2.80)</td>
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</tr>
<tr>
<td>CT w/wo RT</td>
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<td></td>
</tr>
<tr>
<td>GG or AG</td>
<td>66</td>
<td>1.00d</td>
<td>0.28</td>
<td>2.79 (1.01-7.67)</td>
<td>0.047</td>
<td>2.56 (0.94-6.96)</td>
<td>0.066</td>
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</tr>
<tr>
<td>AA</td>
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<td>1.70 (0.65-4.47)</td>
<td></td>
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</tbody>
</table>

*p-Value was based on Cox proportional analysis; breference value: hazard ratio (HR) adjusted for age, stage, radiation therapy, chemotherapy, and hormonal treatment; creference value: HRs adjusted for age, stage, chemotherapy, and hormonal treatment; dreference value: HRs adjusted for age, stage, and radiation therapy; RFS, relapse-free survival; BCSS, breast cancer-specific survival; OS, overall survival; CI, confidence interval; GG, arginine/arginine; AG, glutamine/arginine; AA, glutamine/glutamine; RT, radiation therapy; CT, chemotherapy; HT, hormonal treatment; w/wo, with/without; bold type indicates statistical significance.
concomitant chemotherapy and radiotherapy (n=102), or chemotherapy only (n=77), the rs25487 AA genotype was reported to confer improved DFS (27). In a subgroup analysis of this study, the variant AA genotype was associated with superior DFS and OS in 116 patients treated with neoadjuvant or adjuvant chemotherapy. It is worth noting that the OS in this study was defined as the time between primary surgery and death caused by cancer. There are also studies in patients with breast cancer that did not find any significant prognostic associations between rs25487 genotype and survival (24, 28, 29).

The discrepancies in the results of clinical studies plausibly reflect the fact that the repair of DNA damage is a complex process, requiring multiple steps and the interaction of several genes. There is also wide variability in the types and stages of cancer, and in the treatments patients received. One explanation for the discordant results might be allelic imbalance between the germline cells and the tumour. This question has been addressed in a study of CRT-treated patients with esophageal adenocarcinoma (30); no significant allelic imbalance was detected at the rs25487 SNP. To our knowledge, there are no studies reporting the rate of allelic imbalance at this particular locus in breast cancer.

This study is limited by the relatively small sample size and incomplete data regarding the estrogen and progesterone receptor status. However, the percentage of the patients with missing hormone receptor status is fairly small, and the hormone receptor status was not significantly associated with outcome in any of the multivariate survival analyses in different treatment subgroups.

In the current study, there was also worse BCSS among patients homozygous for the A allele in the survival analysis of all the eligible patients. However, since survival did not differ significantly in the cohort of patients who did not receive any kind of adjuvant treatment (n=140), this finding might reflect the influence of the rs25487 polymorphism on the outcome of patients treated with radiotherapy or chemotherapy.

Conclusion

In conclusion, we provide evidence that the XRCCI rs25487 polymorphism may influence the clinical outcome of patients with breast cancer, especially those treated with postoperative radiotherapy and adjuvant chemotherapy. Therefore, there clearly is a need for further functional and epidemiological studies to elucidate the role of the polymorphisms in DNA repair genes in order to develop tools for individualizing cancer treatments.

Conflict of Interest Statement

The Authors declare no conflicts of interest.

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