

# Polymorphism of XRCC3 in Egyptian Breast Cancer Patients

This article was published in the following Dove Press journal:  
*Pharmacogenomics and Personalized Medicine*

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**Purpose:** Polymorphisms of DNA repair genes may contribute to variations in DNA repair capacity and subsequent genetic susceptibility to different cancers. In Egypt, breast cancer is the most common cancer among women, representing 18.9% of the total cancer cases. The present study assesses the correlation between X-ray repair cross-complementing group 3 (*XRCC3*) polymorphism with breast cancer and treatment response in Egyptian female breast cancer patients.

**Patients and Methods:** This pilot case-control study was conducted on 66 female breast cancer patients and 20 apparently healthy females as a control group. Tumor grading, immunohistostaining of hormone (progesterone and estrogen) receptors and human epidermal growth factor receptor 2 (HER2), and RFLP-PCR for *XRCC3* (rs861539) polymorphism were performed. All breast cancer patients received a treatment protocol (after surgery) which was either chemotherapy (anthracyclines followed by paclitaxel or anthracyclines + fluorouracil) or radiotherapy, or both. Disease-free survival (DFS) and overall survival (OS) were recorded.

**Results:** The number of patients with a heterozygous allele (GA) was significantly higher in cases of tumor size >20 mm. The A allele was correlated with younger age at diagnosis in both chemotherapy and radiotherapy groups. Poor treatment response and higher mortality rates were significantly associated with AA and GA compared with GG alleles (normal allele). In the chemotherapy group, out of eight patients with the A allele, six showed a poor response to treatment containing fluorouracil.

**Conclusion:** *XRCC3* rs861539 polymorphism could be associated with lower DFS and OS and poor treatment response. So, we recommend carrying out *XRCC3* genotyping before starting treatment to choose the most effective treatment strategy according to *XRCC3* polymorphism.

**Keywords:** breast cancer, gene polymorphism, *XRCC3*, treatment response, survival

## Introduction

Breast cancer (BC), a malignant proliferation of the epithelial cells that line the ducts or lobules of the breast, is the most common malignancy in women, accounting for approximately one-third of all cancers in women worldwide.<sup>1</sup> According to its death rate, it is the second most frequent cancer causing mortality for women worldwide.<sup>2</sup> In Egypt, the prevalence of BC in the years 2008–2011 in Upper, Lower, and Middle Egypt was 38.7%, 33.8%, and 26.8%, respectively.<sup>3</sup>

BC is a disease with multifactorial genetic, environmental, reproductive, and lifestyle-related factors that influence disease formation.<sup>4</sup> DNA repair and cell cycle control mechanisms maintain genomic stability. When DNA damage occurs, DNA

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repair pathways, cell cycle arrest, and apoptosis may be activated.<sup>5</sup> Therefore, single-nucleotide polymorphisms (SNPs) in DNA repair genes may alter an individual's capacity to repair damaged DNA and may lead to genetic instability and contribute to malignant transformation.<sup>6</sup>

The *X-ray repair cross-complementing group 3 gene* (*XRCC3*) belongs to a family of genes responsible for homologous recombination, repairing DNA double-strand breaks (DSBs) and interstrand cross-links caused by normal metabolic processes and exposure to ionizing radiation.<sup>7</sup> The *XRCC3* gene is structurally and functionally related to the *RAD51* gene, which is known to play an important role in all three stages of homologous recombination and catalyzes the invasion of broken ends of the DSB into the intact sister chromatid. Moreover, *XRCC3* takes part in DSB repair as it causes slowing of DNA synthesis and recruitment of RAD51 at repair sites.<sup>8</sup>

Several studies have been performed to evaluate the relationship between the rs861539 G/A polymorphism (also named Thr241Met) of the *XRCC3* gene and cancer risk, making it the most commonly studied polymorphism of the *XRCC3* gene.<sup>2</sup> A series of *XRCC3* association trials have yielded controversial results, yet a meta-analysis suggests that common *XRCC3* polymorphisms are associated with the BC risk.<sup>9</sup> Furthermore, another meta-analysis suggests that the Thr241Met polymorphism confers a weakly increased BC risk.<sup>10</sup> Several studies observed a wide variation in treatment response in female BC patients despite nearly the same clinical circumstances, including staging of BC, surgical removal, and treatment after surgery.<sup>11</sup> Also, other studies concluded that SNPs in DNA repair and cell cycle control genes are associated with clinical outcome in many cancers. *XRCC3* (rs861539) polymorphism was reported to affect treatment response and clinical outcomes.<sup>12</sup> So, we studied the association between *XRCC3* (rs861539) gene polymorphisms and the risk of poor prognosis of BC in Egyptian women, and also the effect of these polymorphisms on the treatment response by estimating disease-free survival (DFS) and overall survival (OS) after treatment.

## Patients and Methods

### Ethical Considerations

This study was carried out in accordance with the Declaration of Helsinki for experiments involving humans, and its protocol was reviewed and approved by Al-Azhar University Faculty of Pharmacy (Girls) Institutional

Review Board (approval no. 51). Written informed consent was submitted by all subjects when they were enrolled.

### Study Design

This study was carried out at Al-Azhar University Hospital (Damietta) from July 2016 to December 2019. A total number of 86 participants were enrolled in this study: 66 Egyptian women newly diagnosed with BC; and 20 age-matched apparently healthy females, with no history of health problems, normal routine checks, and comparable socioeconomic factors, as a control group.

Medical history, demographics, age at menarche, age at delivery of first child, number of children, age at menopause, hormone replacement therapy (HRT), and family history were obtained for every participant (patients and controls), and we compared these parameters between controls and BC patients through a case-control study. For BC patients, another cross-sectional study was conducted by collecting additional information and examination results, including age at diagnosis, tumor grading, tumor metastasis, tumor size, lymph-node metastases, type of treatment, DFS, and OS. Immunohistostaining of hormone (progesterone and estrogen) receptors and human epidermal growth factor receptor 2 (HER2), and restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) for *XRCC3* (rs861539) polymorphism were performed. All BC patients were followed up to a maximum 40 months (the duration of our study). The diagnosis of BC was confirmed by histopathologic analysis. Patients with severe clinical symptoms or recurrent cancer were excluded from this study.

Treatment protocols (after surgery) in the study population were either chemotherapy (anthracyclines followed by paclitaxel or anthracyclines + fluorouracil) or radiotherapy, or both. DFS and OS were recorded.

### Immunohistochemical Assay Procedure

Monoclonal antibody 1D5 was used to identify estrogen receptors (ERs) in patients (M7047; DakoCytomation, Carpinteria, CA). For cytologic smears, the immunohistochemical procedure for ERs was identical to the procedure used for the histologic slides and did not require destaining of the smears. In cytologic specimens, no immunohistochemical analysis for progesterone receptors (PRs) was carried out. HER2 staining was performed using the Ventana iVIEW DAB Detection Kit. The staining procedure using this kit is based on the indirect biotin streptavidin system. The heat antigen recovery protocol was used for paraffin-embedded sections as recommended by

the manufacturer. The primary antibody, the rabbit monoclonal Ventana I-V primary antibody (4B5), was used for PATHWAY (Roche Diagnostics) anti-HER 2/neu. The main antibody was the primary antibody.

## DNA Extraction

We collected 5 mL blood samples by venepuncture in Vacutainer tubes from all subjects. DNA (genomic) was extracted using the Quick-gDNAMiniPrep kit (Zymo Research Corporation, Valencia, CA; catalogue nos. D3024, D3006, and D3025) following the manufacturer's instructions. The eluted DNA was stored at  $\leq -20^{\circ}\text{C}$  for future use.

## XRCC3 Genotyping

*XRCC3* (rs861539) gene polymorphism was determined by RFLP-PCR, using a ready-made assay kit with codon 241 primers (GENETAQ Green PCR Master Mix; Genetix Company, India; catalogue no. 108A/B).

Thermal cycling was performed as follows: initial activation at  $95^{\circ}\text{C}$  for 12 minutes, followed by 30 amplification cycles consisting of denaturation at  $95^{\circ}\text{C}$  for 30 seconds, annealing at  $64^{\circ}\text{C}$  for 30 seconds, and extension at  $72^{\circ}\text{C}$  for 1 minute, followed by a final extension at  $72^{\circ}\text{C}$  for 10 minutes. After thermal cycling, the PCR product was run on a 3% agarose gel alongside a 50 bp ladder.

RFLP was performed using Tango Buffer (Thermo Scientific, Sigma Co., UK). For each sample, 10  $\mu\text{L}$  of the PCR amplicons (0.1–0.5  $\mu\text{g}$  of DNA) was digested with 5  $\mu\text{L}$  Tango digest. Then, 2  $\mu\text{L}$  of Tango Buffer, 1–2  $\mu\text{L}$  NOCI, and 18  $\mu\text{L}$  of nuclease-free water were added to the previous mixture. The mixture was mixed gently, spun down for a few seconds, centrifuged, and then incubated at  $37^{\circ}\text{C}$  in a thermostat for 1–6 hours. The digestion products were ready to separate on a 3% agarose gel. The gel was visualized on an ultraviolet transilluminator.

## Statistical Methodology

Statistical analysis was performed using SPSS version 23.0 (IBM Corp., Armonk, NY). Continuous variables were expressed as mean  $\pm$  SD (standard deviation). Student's *t*-test was used for analysis. The differences in allelic and genotypic frequencies between the case and control groups were estimated by Pearson's chi-squared test.

Patients were grouped according to age at diagnosis ( $<45$  and  $\geq 45$  years), tumor size grade (grades T1, T2, T3, and T4), menstrual status (premenopausal and postmenopausal), hormonal receptor status (ER, PR, and HER2), and allele genotype frequency differences.

The frequency deviations of the genotype were assessed for *XRCC3* (rs861539) polymorphism, and the chi-squared standard was used to compare the Hardy–Weinberg equilibrium with control values. Genotype frequencies were compared with the controls in the test cases by the chi-squared test. Specific genotype risks were estimated by unconditional logistic regression as odds ratios (ORs) with associated 95% confidence intervals (CIs).

Multiple logistic regression analysis was performed, adjusted for age, body mass index BMI, menstrual status, marital status, history of BC in the family, age at menarche, number of pregnancies, and history of HRT.

A Cox proportional hazards regression model was used to estimate the hazard ratio. Both OS and DFS were calculated using the Kaplan–Meier method and compared with a log-rank test at a statistical significance level of  $P < 0.05$ .

## Results

### Demographic and Clinical Data

Our study comprised 66 confirmed newly diagnosed BC cases with a mean age of  $48.27 \pm 10.18$  years compared with 20 apparently healthy control subjects with a mean age of  $48.60 \pm 9.35$  years. Thirty-six (54.5%) of the patients with BC were treated with chemotherapy, 25 (37.9%) were treated with radiotherapy, and five (7.6%) underwent concomitant radiotherapy with chemotherapy. The mean DFS of enrolled patients was  $27.18 \pm 11.59$  months, ranging from 6 to 40 months (duration of study), with a mortality rate of 4.5%.

The number of patients with a heterozygous allele (GA) was significantly higher in cases of tumor size  $>20$  mm. The A allele was correlated with younger age at diagnosis in both chemotherapy and radiotherapy groups. Poor treatment response and higher mortality rates were significantly associated with AA and GA compared with GG alleles. In the chemotherapy group, out of eight patients with the A allele, six showed a poor response to treatment containing fluorouracil.

### Genotyping of XRCC3

Among the 66 BC patients and 20 controls, 51 BC patients and 14 controls had the GG genotype, BC patients and five controls had the GA genotype, and lastly two BC patients and one control had the AA genotype (Table 1).

With regard to frequencies of genotype *XRCC3* (rs861539) polymorphism and the clinical status of the studied patients, no statistically significant differences were observed in genotype frequencies of *XRCC3* (rs861539) polymorphism and menstrual status of BC patients ( $P=0.607$ ), tumor size grade ( $P=0.646$ ), lymph-node enlargement ( $P=0.455$ ), or distant metastasis ( $P=0.686$ ). Regarding tumor size, cases with the AA allele were not suitable for statistical analysis owing to their low number ( $n=2$ ). The number of patients with a heterozygous allele (GA) was significantly higher in cases of tumor size  $>20$  mm compared to those with tumor size  $\leq 20$  mm. With regard to reaction to ER, PR, and HER2, no statistically significant difference was observed in genotype frequencies of *XRCC3* rs861539 polymorphism in BC patients. Shorter DFS and higher mortality rates were observed in those with GA alleles compared to the GG genotype allele ( $***P<0.001$  and  $*P=0.0415$ ), respectively (Table 2).

## Treatment Response

In both the chemotherapy and radiotherapy groups, mean age at diagnosis was significantly lower in patients with a heterozygous allele (GA) than in those with the GG allele. Also, in the chemotherapy group, but not the radiotherapy group, the number of premenopausal cases was significantly lower in patients with the A allele compared to those with the GG genotype (Table 1).

Of the 66 BC patients, there were 17 (25.7%) triple-negative patients. The genotype frequency of the studied SNPs was calculated in the triple-negative group and compared to the results obtained in the normal controls. Genotype and allele frequencies of *XRCC3* (rs861539) were tested assuming two models of allele dominance in the triple-negative patients. Allele distribution did not differ significantly between triple-negative BC and controls, either when assuming that G was dominant allele or when assuming that A was the dominant allele (Table 1).

In a Cox proportional hazards model that included menstrual status, tumor size, distant metastases, tumor receptors, and *XRCC3* (rs861539), tumor size, distant metastases, and *XRCC3* (rs861539) polymorphism were strong independent predictors of DFS and OS (Table 3).

For the *XRCC3* (rs861539) polymorphism, irrespective of type of treatment, poor treatment response (represented by DFS) was significantly associated with AA and GA

alleles compared with carriers of GG alleles (Figure 1). DFS in A allele carriers was significantly lower than in patients with the GG genotype in both chemotherapy and radiotherapy groups ( $***P<0.01$ ).

In the chemotherapy group, out of eight patients with the A allele, six showed a poor treatment response to the FAC treatment regimen.

Higher mortality rates were observed in patients with GA alleles compared to the GG genotype allele ( $***P<0.001$  and  $*P=0.0415$ , respectively). From these results, we conclude that *XRCC3* rs861539 polymorphism could be associated with lower DFS and OS, and poor treatment response (Figure 1).

## Discussion

BC is a genetic disease characterized by high cure rates with early diagnosis.<sup>1</sup> Examinations of subjects with high genetic risk would facilitate early diagnosis, eventually leading to reduced mortality rates.<sup>5</sup> Several studies have shown significant associations between *XRCC3* polymorphism and an increased risk of colon cancer,<sup>13</sup> gastric cancer,<sup>14</sup> bladder cancer,<sup>15</sup> thyroid cancer,<sup>16</sup> renal cell carcinoma,<sup>17</sup> and lung cancer.<sup>18</sup>

In the present study, the DNA repair pathway *XRCC3* gene polymorphism was studied to assess its impact on clinical outcomes. Both univariate and multivariate logistic regression analyses were carried out after adjustment for reproductive factors. However, the present case-control study did not reveal any significant link between *XRCC3* (Thr241Met; rs861539) polymorphism and the risk of incidence of BC in females from the Damietta region of Egypt.

Our results are in line with the results of several previous studies.<sup>2,4,8,12,19,20</sup> They showed no association of the *XRCC3* (rs861539) gene with the risk of BC and thus were compelled to hypothesize that ethnic variation persists between *XRCC3* polymorphism and the risk of BC in females.

On the other hand, some studies reported that the AA genotype in the *XRCC3* (rs861539) gene significantly increased the risk of BC.<sup>9–11,21</sup> In a meta-analysis of 23 case-control studies, Chai et al<sup>21</sup> reported an association between the mentioned polymorphism and BC risk, especially in Asian populations and in patients without a family history of BC.

In a study published in 2019, patients carrying the *XRCC3*-rs861539 AA genotype (241 Met/Met) had an increased risk of progression compared with the GA and

**Table I** Distribution of Genotype Alleles in Patients with Breast Cancer and Controls

Genotype XRCC3 (rs861539)	Total Participants		OR (95% CI)	P-value
	Cases (N=66)	Controls (N=20)		
GG	51 (77.3%)	14 (70.0%)	Ref	
GA	13 (19.7%)	5 (25.0%)	1.40 (0.42–4.60)	0.445
AA	2 (3.0%)	1 (5.0%)	1.82 (0.15–21.6)	0.925
	Age at diagnosis <45 years		OR (95% CI)	P-value
	Cases (N=33)	Controls (N=9)		
GG	25 (75.8%)	7 (77.8%)	Ref	
GA	6 (18.2%)	2 (22.2%)	1.19 (0.19–7.25)	0.776
AA	2 (6.0%)	0 (0.0%)	1.78 (0.14–22.7)	0.874
	Age at diagnosis ≥45 years		OR (95% CI)	P-value
	Cases (N=33)	Controls (N=11)		
GG	26 (78.8%)	7 (63.6%)	Ref	
GA	7 (21.2%)	3 (27.3%)	1.59 (0.32–7.80)	0.881
AA	0 (0.0%)	1 (9.1%)	1.71 (0.20–6.71)	0.526
	Participants		OR (95% CI)	P-value
	Triple-negative cancer (N=17)	Controls (N=20)		
GG	13 (76.5%)	14 (70.0%)	Ref	
GA	3 (17.6%)	5 (25.0%)	1.40 (0.42–4.60)	0.267
AA	1 (5.9%)	1 (5.0%)	0.95 (0.35–2.58)	0.910
GA+AA	4 (23.5%)	6 (30.0%)	1.35 (0.63–2.88)	0.292
GA+GG	16 (94.1%)	19 (95.0%)	11.09 (0.65–0.05)	0.22
	Chemotherapy			P-value
	Age at diagnosis, mean±SD			
GG	46.79±10.84			0.048*
GA+AA	38.5±6.37			
	Radiotherapy			P-value
	Age at diagnosis, mean±SD			
GG	52.86±10.45			0.033*
GA	43.06±7.03			
	Chemotherapy		Chi-square	P-value
	Premenopausal	Postmenopausal		
GG	13	15	5.622	0.017*
GA+AA	7	1		

(Continued)

**Table 1** (Continued).

Genotype <i>XRCC3</i> (rs861539)	Total Participants		OR (95% CI)	P-value
	Cases (N=66)	Controls (N=20)		
	Radiotherapy		Chi-square	P-value
	Premenopausal	Postmenopausal		
GG	12	6	0.405	0.524
GA	3	4		

Note: \*P-value <0.05 and indicates significance.

**Table 2** Frequencies of Genotype *XRCC3* (Rs861539) With Regard to Clinical Status of Studied Patients

Patient Status		Genotype SNPs		Chi-Square	P-value
		GA+AA	GG		
Menstrual status	Postmenopausal	5	23	0.263	0.607
	Premenopausal	10	28		
Tumor size (mm)	≤20	6	39	5.255	0.018*
	>20	9	12		
Tumor size grade of breast cancer	T1	6	18	1.656	0.646
	T2	7	28		
	T3	2	3		
	T4	0	2		
Lymph-node involvement	No	12	40	0.557	0.455
	Yes	1	11		
Distant metastasis	No	13	48	0.163	0.686
	Yes	2	3		
Estrogen receptors	Negative	8	35	0.616	0.432
	Positive	7	16		
Progesterone receptors	Negative	10	44	1.823	0.177
	Positive	5	7		
Human epidermal growth factor receptor 2	Negative	7	23	0.022	0.989
	Positive	8	28		
Type of treatment	Chemotherapy	8	28	1.872	0.392
	Radiotherapy	7	18		
	Chemotherapy and radiotherapy	0	5		
Disease-free survival	<20 months	15	10	31.83	<0.001***
	20–30 months	0	7		
	30–40 months	0	34		
Overall survival	Survival	12	51	6.573	0.010*
	Dead	3	0		

Notes: \*P-value <0.05 and indicates significance. \*\*\*P-value <0.001 and indicates high significance.

GG genotypes. The 241 Met/Met variant was associated with a decreased DNA repair capacity and it has been considered a risk for BC.<sup>11</sup>

Regarding a link between incidence of BC and disease risk factors, there were no significant differences between the studied groups with regard to age, BMI, marital status, family

**Table 3** Multivariable Analysis of DFS and OS

Demographic Feature	DFS		OS	
	HR	95% CI	HR	95% CI
Menstrual status	0.60	0.39 to 0.93	0.68	0.44 to 1.05
Tumor size (mm)	2.3	1.27 to 4.16	1.6	0.94 to 2.96
Distant metastasis	2.3	1.27 to 4.16	2.4	1.3 to 4.48
Triple-negative cancer	1.6	0.97 to 2.54	1.5	0.94 to 2.51
<i>XRCC3</i> (rs861539)	2.4	1.27 to 4.34	2.7	1.49 to 4.97

**Abbreviations:** DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.

history of BC, onset of menarche, menstrual status, or history of HRT ( $P>0.05$ ), except that BC patients in our study had a statistically significantly lower number of pregnancies compared to the control group.

Another study found no statistically significant differences in BMI, number of children, receiving HRT, or alcohol consumption history, while there were significant differences in age, smoking, age at menarche, age at first delivery, and family history between the two groups.<sup>4</sup>

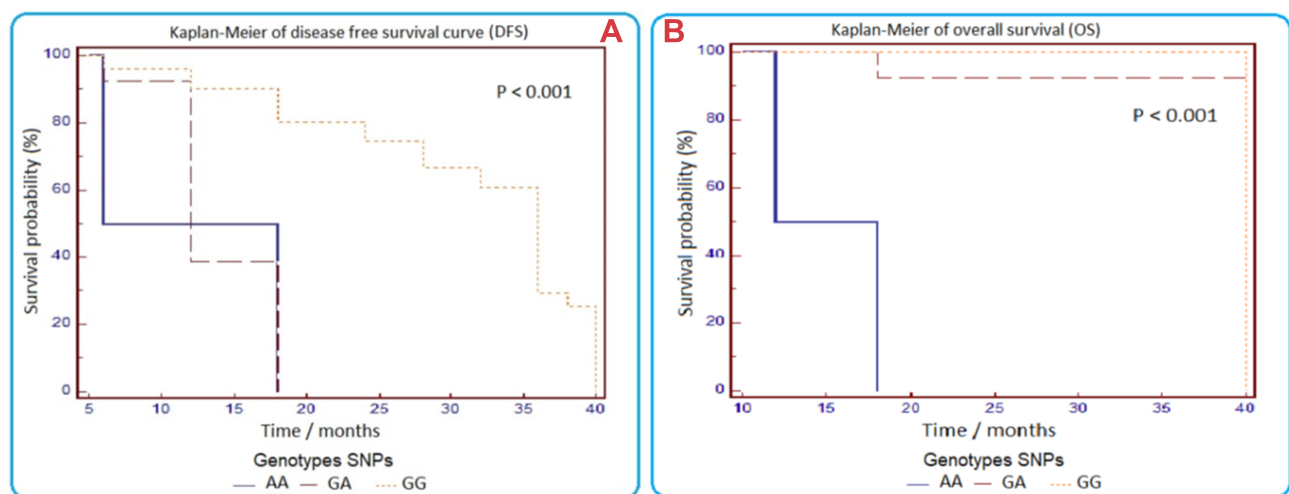
For the analysis of age of onset of BC, our study showed that in both the chemotherapy and radiotherapy groups the mean age at diagnosis was significantly lower in patients with the heterozygous allele (GA) than in those with the GG allele. Also, in the chemotherapy group (but not the radiotherapy group), the number of premenopausal cases was significantly higher in patients with the A allele compared to those with the GG genotype, which could suggest a role of this allele in the early onset of BC.

These results are in agreement with Ali et al,<sup>12</sup> who found that the A allele predisposes to BC at a younger age.

In the current study, *XRCC3* (rs861539) polymorphism was not related to BC risk in clinical samples. However, nucleotide variation was shown to affect its expression in TCGA PanCan 2018 dataset, and analysis of the METABRIC dataset revealed significant coexpression between *XRCC3* and both *BRCA1* and *BRCA2*, which are indicators of BC risk. This could be explained as the coexpression, despite being significant, showed a weak association ( $r<0.3$  and  $0.1$  respectively). This weak association could be more detectable in large studies such as METABRIC than in small-scale studies.

Studies that revealed that polymorphisms in the *XRCC3* (rs861539) gene were significantly associated with an increased risk of BC were conducted in different ethnic populations, and the results are inconsistent with our findings, which may be due to environmental factors, ethno-cultural variations, and/or variations in linkage disequilibrium of the *XRCC3* gene.<sup>19,22,23</sup>

Regarding tumor size, the number of patients with a heterozygous allele (GA) was significantly higher in cases with tumor size  $>20$  mm compared to those with



**Figure 1** Kaplan-Meier curves of disease-free survival (A) and overall survival (B) in patients with breast cancer according to *XRCC3* (rs861539) polymorphism.

tumor size  $\leq 20$  mm. This could also suggest a role of this allele in tumor growth. These results are in agreement with a previous study by Ali et al,<sup>12</sup> whose results suggested the involvement of the A allele in disease severity.

In the current study, analysis of the study samples showed no significant correlation between *XRCC3* (rs861539) polymorphism and any of ER, PR, or HER2, or triple-negative BC. Also, analysis of GDS4053 data revealed no correlation between *XRCC3* expression and expression of ER-alpha, PR, and HER2, while ER-beta was significantly correlated with *XRCC3* expression. These results are comparable to those of Özgöz et al,<sup>4</sup> who reported no significant correlation between *XRCC3* (rs861539) polymorphism and hormone receptor status, although they reported a significant correlation with HER2 negativity.

In the current study, for the *XRCC3* (rs861539) polymorphism, irrespective of treatment regimen, survival analysis showed a significantly lower OS and DFS for patient carriers of the AA and GA alleles compared with carriers of GG alleles. These results are in line with previous studies<sup>12,19,20,23,24</sup> which reported a significant association between *XRCC3* (rs861539) polymorphism and both DFS and 5–10-year OS. In contrast to our results, Pelttari et al<sup>8</sup> performed 10-year BC-specific survival analyses for the *XRCC2* p.(Arg188His) missense variant as well as the *XRCC3* (rs861539) variant, which were both detected in the sequencing of the genes and also included in the haplotype analysis. They found no survival effect for *XRCC3* (rs861539) variants.

Poor response to the treatment regimen containing 5-fluorouracil was observed in the current study in patients with the A allele (GA and AA), although the small group number did not allow for proper statistics. The effect of *XRCC3* (rs861539) polymorphism on the response to 5-fluorouracil and other chemotherapeutics needs more studies.

The role of *XRCC3* protein in BC prognosis and treatment response could be related to proteins interacting with it. In this context, we analyzed GDS4053 for *XRCC3* functional protein network members, and a significant positive correlation was found between *XRCC3* expression and RAD51, RAD51C, ATM, and MKI6. These results are in agreement with Hu et al,<sup>7</sup> who reported a strong association between *XRCC3* expression and RAD51 (and its homolog RAD51C) expression. ATM signaling pathways were shown to play an important role in homologous recombination and epithelial–mesenchymal transition (EMT). Improper ATM signaling has been suggested to cause increased BC risk.<sup>25</sup> Regarding MKI67, a previous study showed that MKI67 mRNA

expression was an independent predictor of distant DFS.<sup>26</sup> This, together with the significant correlation between *XRCC3* and KI67, could support the results of the current study linking *XRCC3* (rs861539) polymorphism with DSF.

Those genes that were found to correlate significantly with *XRCC3* expression are mostly related to poor prognosis in cancers. POLQ overexpression is present and is associated with poor prognosis in many cancer types, including BC, regardless of the *BRCA1* status.<sup>27</sup> CDCA4 had been reported to be involved in drug resistance in BC as well as in disease progression in triple-negative BC.<sup>28</sup> ASF1a overexpression is known to be widespread in human cancers, including BC, as it is required for the proliferation of cancer cells.<sup>29</sup> Also, TROAP plays an important role in promoting proliferation, invasion, and metastasis of BC owing to its role in mediating spindle assembly and centrosome integrity. Hence, it could be a marker for the prediction of poor prognosis in BC.<sup>30</sup> TEDC1 is a transcriptional target of NEK6, the signaling of which was reported to mediate drug resistance in prostate cancer.<sup>31</sup> PKMYT1 was reported to positively regulate the growth, migration, metastasis, and EMT of hepatocellular carcinoma cells. Mechanistically, it acts mostly by activating beta-catenin/TCF signaling.<sup>32</sup> Overexpression of MCMs (especially MCM2, MCM5, and MCM7) was found to be significantly correlated with poor prognosis in many cancers, including BC.<sup>33</sup> Taken together, the correlation between *XRCC3* expression and these genes could contribute to its role in treatment response and drug resistance.

## Conclusion

*XRCC3* (rs861539) could have a significant association with treatment response (anthracyclines followed by paclitaxel, anthracyclines + fluorouracil, and radiotherapy) in the form of lower DFS and OS for patient carriers of the AA and GA alleles. The presence of the A allele could be a predictor of DFS. The present study did not reveal any significant association of *XRCC3* (rs861539) polymorphism with the clinical parameters of BC risk. *XRCC3* expression correlated significantly with *BRCA1* and *BRCA2* expression, along with several genes related to cell proliferation, cancer progression, and treatment response.

## Abbreviations

BC, breast cancer; BMI, body mass index; *BRCA1*, breast cancer susceptibility gene 1; *BRCA2*, breast cancer susceptibility gene 2; DFS, disease-free survival; DSB,



double-strand break; EMT, epithelial–mesenchymal transition; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HRT, hormone replacement therapy; OR, odds ratio; OS, overall survival; SD, standard deviation; SNP, single-nucleotide polymorphism; *XRCC3*, X-ray repair cross-complementing group 3 gene.

## Disclosure

The authors report no conflicts of interest in this work.

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