

Association of the *XRCC1* gene polymorphisms with cancer risk in Turkish breast cancer patients

Ugur Deligezer¹ and Nejat Dalay^{1,2}

¹Department of Basic Oncology
Oncology Institute Istanbul University
Istanbul, Turkey

²Corresponding author: Tel, 90-212-5313100;
Fax, 90-212-5348078; E-mail, ndalay@yahoo.com

Accepted 9 December 2004

Abbreviations: BRCA1, Breast cancer-associated gene 1; BRCT-1, BRCA1 C terminus repeat 1; CI, Confidence interval; OR, Odds ratio; *XRCC1*, X-ray repair cross-complementing group 1

Abstract

The X-ray repair cross-complementing group 1 (*XRCC1*) gene is believed to play an important role in base excision repair and displays genetic polymorphisms. Data on the role of *XRCC1* polymorphisms in cancer susceptibility is inconsistent. In the present study, we investigated the effect of two *XRCC1* polymorphisms, Arg194Trp and Arg399Gln, on breast cancer risk in a case-control study involving Turkish breast cancer patients and healthy women. Both alleles exhibited a similar distribution among cases and controls leading to lack of any significant association between the *XRCC1* polymorphisms and breast cancer risk, either in homozygotes and heterozygotes or combined. The allele frequency of the codon 194 variant was very low in cases and healthy individuals (5.3 and 3.9%, respectively) compared to that of the variant 399Gln allele (39.7 and 37.4%). Our results do not support evidence for a role of the *XRCC1* polymorphism in developing breast cancer.

Keywords: breast cancer; risk; polymorphism; *XRCC1*

Introduction

Breast cancer is the most prevalent common malignancy among women and the incidence is still increasing. A substantial fraction of breast cancer cases is explained by well-established risk factors such as the age at the first child's birth, nulliparity

and family history (Madigan *et al.*, 1995). In the majority of cases the cause of the disease is still obscure. Amino acid substitutions in the DNA repair genes as result of genetic polymorphisms may lead to alterations in DNA repair capacity and affect the susceptibility to cancer. Polymorphic alleles have been described for many DNA repair genes including the genes responsible for nucleotide and base excision repair (Shen *et al.*, 1998; Fan *et al.*, 1999). Recent data suggest that DNA repair capacity may vary between individuals (Berwick, 2000) and is lower in cancer patients than healthy controls (Mohrenweiser and Jones, 1998). It has been hypothesized that multiple alleles may act in combination in conferring cancer risk (Mohrenweiser and Jones, 1998; Shen *et al.*, 1998).

The protein encoded by the *XRCC1* gene plays an important role in base excision repair and removes base adducts formed by ionizing radiation and alkylating agents (Yu *et al.*, 1999). The multi-domain protein interacts with DNA ligase III, DNA polymerase β and poly (ADP-ribose) polymerase in the base excision repair pathway (Kubota *et al.*, 1996; Nash *et al.*, 1997; Masson *et al.*, 1998; Vidal *et al.*, 2001). It contains a BRCT-1 domain bearing homology to *BRCA1* through which it interacts with DNA ligase III (Kubota *et al.*, 1996) and poly (ADP-ribose) polymerase (Masson *et al.*, 1998). Three polymorphisms (Arg194Trp, Arg280His and Arg399Gln) in the *XRCC1* gene have been described (Mohrenweiser and Jones, 1998). The Arg399Gln polymorphism occurs within the BRCT-1 domain while the Arg194Trp leads to an amino acid substitution in the hydrophobic region of the protein. Since amino acid substitutions in the active sites may result in reduced efficiency to repair DNA damage these polymorphisms may confer increased risk to breast cancer. Concordantly, the codon 399 polymorphism has been shown to affect DNA repair capacity (Lunn *et al.*, 1999), while the significance of the other two polymorphisms is not yet known.

Previous studies investigating the association between genetic polymorphisms and risk of different cancer types have provided inconsistent results (Ratnasinghe *et al.*, 2001; Stern *et al.*, 2001; Nelson *et al.*, 2002). Data from studies investigating the association of the *XRCC1* polymorphisms with breast cancer risk are also inconsistent (Duell *et al.*, 2001; Kim *et al.*, 2002; Moullan *et al.*, 2003; Shu *et al.*, 2003; Smith *et al.*, 2003a; b). In the present study, we investigated the effect of two polymorphisms in the *XRCC1* gene on breast cancer risk in a Turkish breast cancer population.

Materials and Methods

Our study group consisted of breast cancer patients with similar ethnical background ($n = 151$; mean age 51.5 ± 11.2 ; age range 29-75) regardless of menopausal status and family history to evaluate the general cancer risk in unselected cases. Control samples were taken from 133 healthy women (mean age 41 ± 11.3 ; age range 23-70). Genomic DNA was prepared from blood lymphocytes according to the standard protocol.

Genotyping of the *XRCC1* polymorphisms was performed by a multiplex PCR. The reaction mixture contained 2.5 μ l 10X reaction buffer, 2.5 mM $MgCl_2$, 200 μ M dNTPs, 0.6 μ M of each primer, 0.5 μ g DNA and 2.0 U of Taq polymerase (Promega, Madison) in a total volume of 25 μ l. Primers (Integrated DNA Technologies, Coralville, IA) used were; for codon 194: 5'-GCCCCGTCCCAGGTA-3' (forward) and 5'-AGCCCCAAGACCCTTTCCT-3' (reverse) and for codon 399: 5'-TTGTGCTTTCTCTGTGTCCA-3' (forward) and 5'-TCCTCCAGCCTTTTCTGATA-3' (reverse). The initial denaturation of 5 min at 95°C was followed by 35 cycles of amplification beginning with a denaturation step of 30 s at 95°C, annealing for 1 min at 62°C and extension for 45 s at 72°C. The reaction was completed by a final extension for 5 min at 72°C. The products (491 bp and 615 bp for codons 194 and 399, respectively) were resolved on a 1.5% agarose gel containing 5 μ g/ml ethidium bromide and visualized under UV-light.

Enzyme digestions of the PCR products were performed in a final volume of 20 μ l at 37°C for 16h using 5 U of *Hpa*II (Promega, Madison). The fragments were separated on 1.5% agarose gels containing ethidium bromide and evaluated using a video gel documentation system (Vilber-Lourmat, Cedex, France).

The enzyme *Hpa*II recognizes the wild-type alleles of codons 194 and 399. It has two recognition sites on the 491 bp fragment at the positions 174 and 198 of which the position 198 is the polymorphic site. Thus, the wild-type allele of codon 194 generates three fragments of 292, 174 and 24 bp, while the variant allele leads to two fragments of 318 and 174 bp. The 174 bp fragment from the digestion of the 491 bp fragment is always present irrespective of the genotype and was used as an internal control for complete digestion.

The enzyme has a single recognition site on the 615 bp fragment. The wild-type allele results in two fragments of 374 and 241 bp; the variant allele is present as an undigested 615 bp fragment.

Statistical analyses were performed using the statistical package Instat⁺ for Windows (Statistical Services Centre; University of Reading, Reading, UK). Odds ratios (ORs) and 95% confidence intervals were calculated.

Results and Discussion

Genetic polymorphisms in the DNA repair genes may impair their function and enhance cancer susceptibility. The effect of *XRCC1* gene polymorphisms on cancer susceptibility is still not clear and the results up to now are inconsistent (Ratnasinghe *et al.*, 2001; Stern *et al.*, 2001; Nelson *et al.*, 2002). Studies investigating the association between polymorphisms and breast cancer risk have also led to contradictory results. An association between the 399Gln allele and breast cancer risk among African-American (Duell *et al.*, 2001) and Asian women (Kim *et al.*, 2002) has been suggested by two studies. On the other hand, in a more recent report (Smith *et al.*, 2003a) a weak association for the 194Trp allele, but not for the 399Gln allele was observed in Caucasian women. There are also studies in the literature reporting lack of association between the breast cancer risk and 194Trp and/or the 399Gln polymorphisms in Chinese and French breast cancer patients (Moullan *et al.*, 2003; Shu *et al.*, 2003). These results suggest that the risk conferred by *XRCC1* may vary between ethnic groups.

In this study we investigated the association between the two *XRCC1* polymorphisms and breast cancer risk in a homogenous breast cancer population. The frequency of the genotypes was evaluated in breast cancer patients and healthy controls. Figure 1 shows a representative gel for the Arg194Trp and Arg399Gln polymorphisms. The allele frequencies in the healthy controls were consistent with those predicted from the Hardy-Weinberg equilibrium (Table I).

The variant 399Gln allele is much more common than the variant 194Trp allele in both groups. On the other hand, the allele frequency of the codon 194

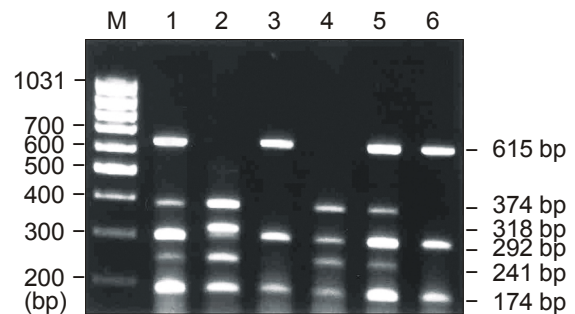


Figure 1. *XRCC1* gene polymorphisms in breast cancer patients. Polymorphic regions of the codons 194 and 399 were amplified by a multiplex PCR, the products were digested with *Hpa*II and separated by gel electrophoresis. Lanes 1 and 5 demonstrate patients with Arg194Arg and Arg399Gln genotypes. Lane 2: Arg194Trp and Arg399Arg; Lanes 3 and 6: Arg194Arg and Gln399Gln; Lane 4: Arg194Arg and Arg399Arg. M is the DNA size marker with indicated fragment sizes left (bp, base pairs).

Table 1. XRCC1 genotypes and allele frequencies in the patients and controls.

XRCC1 194	N	Arg/Arg	Arg/Trp	Trp/Trp	Arg/Trp- Trp/Trp	194Trp frequency
Controls	133	119 (89.5%)	14 (10.5%)	0 (0.0%)	14 (10.5%)	0.053
Breast cancer	151	143 (94.7%) (Reference)	7 (4.6%) <i>P</i> = 0.099 OR = 0.416 95% CI: 0.163-1.064	1 (0.7%)	8 (5.3%) <i>P</i> = 0.155 OR = 0.475 95% CI: 0.193-1.172	0.039
XRCC1 399	N	Arg/Arg	Arg/Gln	Gln/Gln	Arg/Gln- Gln/Gln	399Gln frequency
Controls	133	50 (37.6%)	66 (49.6%)	17 (12.8%)	83 (62.4%)	0.374
Breast cancer	151	58 (38.5%) (Reference)	68 (45.0%) <i>P</i> = 0.742 OR = 0.889 95% CI: 0.534-1.476	25 (16.5%) <i>P</i> = 0.645 OR = 1.2677 95% CI: 0.615-2.61	93 (61.5%) <i>P</i> = 1.0 OR = 0.966 95% CI: 0.598-1.56	0.397

variant was very low in both groups. None of the controls and only one patient harbored the homozygote codon 194 variant. Distribution of both variant alleles was similar among the cases and the controls. The frequency of the variant 194Trp allele among the cases is slightly lower than that in healthy controls while the variant 399Gln allele frequency is slightly higher. Similar distribution of the variant alleles led to lack of any significant association between the XRCC1 polymorphisms and breast cancer risk, either in homozygotes and heterozygotes or combined.

The very low frequency of codon 194Trp/Trp genotype in our patients is in accordance with data reported in Caucasian (0-2%) and African-American (0%) (Duell *et al.*, 2001; Stern *et al.*, 2001; Smith *et al.*, 2003b) patients. The frequency of the codon 399Gln/Gln genotype (12%) is also consistent with the reports on Caucasians (13-16%) (Duell *et al.*, 2001; Stern *et al.*, 2001; Nelson *et al.*, 2002; Smith *et al.*, 2003b). On the other hand, the codon 194Trp/Trp frequency in the Turkish population is considerably lower than that in Asians (13%) (Kim *et al.*, 2002) while the codon 399Gln/Gln frequency is much higher than that of both Asians and African-Americans (Duell *et al.*, 2001; Stern *et al.*, 2001; Kim *et al.*, 2002).

Our results do not provide evidence for an association between the XRCC1 gene polymorphisms and breast cancer risk in the Turkish population. These data are in concordance with the subgroups in the previous studies. Lack of association between the 399Gln allele and breast cancer is in accordance with data on white American women, Chinese and French breast cancer patients (Duell *et al.*, 2001; Moullan *et*

al., 2003; Shu *et al.*, 2003; Smith *et al.*, 2003a) and lung cancer patients from China (Ratnasinghe *et al.*, 2001). Lack of association between the 194Trp allele and breast cancer is also consistent with previous reports (Duell *et al.*, 2001; Moullan *et al.*, 2003). In conclusion, our study suggests that the XRCC1 gene does not provide a susceptibility marker for breast cancer.

Acknowledgement

This work was supported by the Istanbul University Research Fund (Project No: 50/23012003).

References

- Berwick M. Gene-environment interaction in melanoma. *Forum (Genova)* 2000;10:191-200
- Duell EJ, Millikan RC, Pittman GS, Winkel S, Lunn RM, Tse C-KJ, Eaton A, Mohrenweiser HW, Newman B, Bell DA. Polymorphisms in the DNA repair gene XRCC1 and breast cancer. *Cancer Epidemiol Biomarkers Prev* 2001;10:217-22
- Fan F, Liu CP, Tavare S, Arnheim N. Polymorphisms in the human DNA repair gene XPF. *Mutat Res* 1999;406:115-20
- Kim SU, Park SK, Yoo KY, Yoon KS, Choi JY, Seo JS, Park WY, Kim JH, Noh DY, Ahn SH, Choe KJ, Strickland PT, Hirvonen A, Kang D. XRCC1 genetic polymorphism and breast cancer risk. *Pharmacogenetics* 2002;12:335-8
- Kubota Y, Nash RA, Klungland A, Schar P, Barnes DE, Lindahl T. Reconstitution of DNA base excision repair with purified human proteins: interaction between DNA polymerase beta and the XRCC1 protein. *EMBO J* 1996;15:6662-70

- Lunn RM, Langlois RG, Hsieh LL, Thompson CL, Bell DA. XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycoprotein A variant frequency. *Cancer Res* 1999;59:2557-61
- Madigan MP, Ziegler RG, Benichou J, Byrne C, Hoover RN. Proportion of breast cancer cases in the United States explained by well-established risk factors. *J Natl Cancer Inst* 1995;87:1681-5
- Masson M, Niedergang C, Schreiber V, Muller S, Menissier-de Murcia J, De Murcia G. XRCC1 is specifically associated with poly(ADP-Ribose) polymerase and negatively regulates its activity following DNA damage. *Mol Cell Biol* 1998;18:3563-71
- Mohrenweiser HW, Jones IM. Variation in DNA repair is a factor in cancer susceptibility: a paradigm for the promises and perils of individual and population risk estimation? *Mutat Res* 1998;400:15-24
- Moullan N, Cox DG, Angele S, Romestaing P, Gerard JP, Hall J. Polymorphisms in the DNA repair gene XRCC1, breast cancer risk, and response to radiotherapy. *Cancer Epidemiol Biomarkers Prev* 2003;12:1168-74
- Nash RA, Caldecott KW, Barnes DE, Lindahl T. XRCC1 protein interacts with one of two distinct forms of DNA ligase III. *Biochemistry* 1997;36:5207-11
- Nelson HH, Kelsey KT, Mott LA, Karagas MR. The XRCC1 polymorphism, sunburn, and non-melanoma skin cancer: evidence of gene-environment interaction. *Cancer Res* 2002;62:152-5
- Ratnasingham D, Yao SX, Tangrea JA, Qiao YL, Andersen MR, Barrett MJ, Giffen CA, Erozan Y, Tockman MS, Taylor PR. Polymorphisms of the DNA repair gene XRCC1 and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 2001;10:119-23
- Shen MR, Jones IM, Mohrenweiser HW. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. *Cancer Res* 1998;58:604-8
- Shu XO, Cai Q, Gao YT, Wen W, Jin F, Zheng W. A population-based case-control study of the Arg399Gln polymorphism in DNA repair gene XRCC1 and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 2003;12:1462-7
- Smith TR, Miller MS, Lohman K, Lange EM, Case LD, Mohrenweiser HW, Hu JJ. Polymorphisms of XRCC1 and XRCC3 genes and susceptibility to breast cancer. *Cancer Lett* 2003a;190:183-90
- Smith TR, Levine EA, Perrier ND, Miller MS, Freimanis RI, Lohman K, Case LD, Xu J, Mohrenweiser HW, Hu JJ. DNA-repair genetic polymorphisms and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2003b;12:1200-4
- Stern M, Umbach M, van Gils CH, Lunn RM, Taylor JA. DNA repair gene XRCC1 polymorphisms, smoking, and bladder cancer risk. *Cancer Epidemiol Biomarkers Prev* 2001;10:125-31
- Vidal AE, Boiteux S, Hickson ID, Radicella JP. XRCC1 coordinates the initial and late stages of DNA basic site repair through protein-protein interactions. *EMBO J* 2001;20:6530-9
- Yu Z, Chen J, Ford BN, Brackley ME, Glickman BW. Human DNA repair systems: an overview. *Environ Mol Mutagen* 1999;33:3-20