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ORIGINAL PAPER

Polymorphisms of the XRCC1 and XPD Genes and Breast Cancer Risk: A Case-Control Study

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Abstract The purpose of this case control study was to evaluate the role of X-ray repair cross-complementing group 1 (XRCC1) and xeroderma pigmentosum group D (XPD) genotypes as genetic indicators of susceptibility to breast cancer (BC). We analysed DNA samples from 114 breast cancer patients and 113 control subjects using polymerase chain reaction–restriction fragment length polymorphism. For the single nucleotide polymorphisms in XRCC1 exon 10 (Arg399Gln, G/A) and XPD exon 23 (Lys751Gln, A/C), no remarkable differences for genotype distribution and allele frequencies were observed between BC group and control group in the study. The genotype frequency for homozygote A/A in XPD exon 6 (Arg156Arg, C/A) were significantly different between BC and control groups ($P<0.0001$, odds ratio=2.14; 95% confidence interval 1.44–3.17). The data indicate a possible role for *XPD* (Arg156Arg, C/A) polymorphisms in BC susceptibility.

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Introduction

Breast cancer (BC) is the most frequent cancer in women. It represents the second leading cause of cancer death among women [1]. It has been hypothesized that subtle functional deficiencies in highly conserved DNA repair processes resulting from polymorphic variation may increase genetic susceptibility to BC. Polymorphisms in DNA repair genes can impact protein function leading to genomic instability facilitated by growth stimulation and increased cancer risk [2].

DNA repair protein X-ray repair cross-complementing group 1 (XRCC1) is required for the efficient repair of DNA damage caused by ischemia-reperfusion, oxidative stress, and DNA methylating agents. Mutations in XRCC1 result in decreased genetic stability, including increased frequencies of spontaneous or induced chromosome translocations or deletions. Protein XRCC1 has no catalytic activity. However, three domains have been identified within XRCC1 which interact with enzymes and appear to play a pivotal role in base excision repair. Shen et al. [3] identified an amino acid substitution in regions of XRCC1, an arginine to glutamine change at codon 399 (G→A) in exon 10. A number of epidemiological studies have assessed the association of the XRCC1 polymorphism with cancer incidence [4, 5].

Several different complementation groups have been characterized whose protein products take part in the different steps of nucleotide excision repair. Among the repair proteins, the xeroderma pigmentosum group D

(XPD) protein is interesting because it is a major player in the nucleotide excision repair pathway and is also involved in transcription initiation and in the control of the cell cycle and apoptosis [6]. The proteins XPA, XPC and XPE are required for binding to damaged DNA. XPF and XPG are nucleases that, respectively, cleave damaged DNA on the 3′ and 5′ sides of the damage. XPD mutations result in genetic diseases and hence XPD polymorphisms may operate as genetic susceptibility factors [7].

Here, we carried out a case-control study of 114 breast cancer patients and 113 healthy controls. The influence of genotypes on BC incidence has been studied in women from Eastern region of Slovakia. The following single nucleotide polymorphisms (SNPs) in the DNA repair genes were analyzed: XRCC1 exon 10 (Arg399Gln, G/A); XPD exon 6 (Arg156Arg, C/A) and exon 23 (Lys751Gln, A/C).

Material and Methods

Study Population

Blood samples were collected from 114 females with histologically proven diagnosis of BC recruited at the Department of Pathology, University Hospital, Košice, from 2003 to 2005. Blood samples of 113 healthy female donors were collected as controls. The control group was age adjusted according to patients (Table 1). All cases and controls were of Slovak origin (Caucasians) from different regions of Eastern Slovakia. The participation of each subject was voluntary and could be cancelled by any individual at any time during this study (according to the Helsinki II declaration). The local ethics committee approved the study protocol and all volunteers signed the study informed consent form. To determine an individual's life-style (smoking habits, residence, familiar case history), a questionnaire was filled in by each individual.

Table 1 Controls and patients characteristics

Characteristics	Controls	Patients
All subjects (*n*)	113	114
Age (*n*)		
≤50	34	26
≥51	79	88
Range	41–87	41–93
Weight (kg; mean)	71.2	70.4
Height (cm; mean)	165	163
Smokers (*n*)	26	24
No smokers (*n*)	87	90
City (*n*)	87	80
Village (*n*)	26	34
Family case history		
Positive (*n*)	30	52
Negative (*n*)	83	62

DNA Isolation and Genotype Analyses

Genomic DNA was isolated from the peripheral lymphocytes by proteinase K digestion and phenol/chloroform extraction, followed by ethanol precipitation [8].

Oligonucleotide sequences for primers and probes to detect the polymorphisms were:

XRCC1 codon 399: the sense primer P1(5′-TTG TGC TTT CTC TGT GTC CA-3′) and the anti-sense primer P2 (5′-TCC TCC AGC CTT TTC TGA TA-3′) amplified a 615-bp polymerase chain reaction (PCR) product. PCR was performed in a total volume of 50 μL containing 300 ng of genomic DNA, 50 mmol/L KCl, 20 mmol/L Tris–HCl, 1.5 mM mmol/L $MgCl_2$, 200 μmol/L deoxynucleotide triphosphate (dNTP) mix, 50 pmol/L of each primer (Invitrogen, UK), and 1 U Taq-polymerase (Invitrogen, UK). The amplification was carried out for 30 cycles under the following conditions: 1 min at 94°C for denaturation, 1 min at 63°C for primer annealing, and 1 min at 72°C for primer extension. An aliquot of the PCR product (about 10%) was digested with 20 U of *Msp*I restriction enzyme (New England BioLabs, UK) at 37°C overnight (min. 3 h). Electrophoresis was performed on 3% agarose gel for 1.5 h at 150 V, and the DNA fragments were visualized with ethidium bromide.

The Arg→Gln change abolishes the *Msp*I restriction site and the Gln allele does not contain a *Msp*I restriction site. The PCR product is 615-bp in length. The presence of a C$^{\downarrow}$CGG sequence in the Arg allele creates a *Msp*I restriction site that leads to formation of two fragments of 374-bp and 221-bp [9].

XPD exon 23: the sense primer P1(5′-ATC CTG TCC CTA CTG GCC ATT C-3′) and the anti-sense primer P2 (5′-TGT GGA CGT GAC AGT GAG AAA T-3′) amplified a 324-bp PCR product. PCR was performed in a total volume of 50 μl containing 300 ng of genomic DNA, 50 mmol/L KCl, 20 mmol/L Tris–HCl, 1.5 mmol/L $MgCl_2$, 200 μmol/L dNTP mix, 50 pmol/L of each primer (Invitrogen, UK), and 1 U Taq-polymerase (Invitrogen, UK). The amplification was carried out for 30 cycles under the following conditions: 1 min at 92°C for denaturation, 1 min at 56.1°C for primer annealing, and 1 min at 72°C for primer extension. An aliquot of the PCR product (about 10%) was digested with 20 U of *Pst*I restriction enzyme (New England BioLabs, UK) at 37°C overnight (min. 3 h). Electrophoresis was performed on 3% agarose gel for 1.5 h at 150 V, and the DNA fragments were visualized with ethidium bromide.

Lys→Gln (A→C) change abolishes the *Pst*I restriction site. The A but not C allele has *Pst*I restriction site

(CTGCA$^{\downarrow}$G) within 324-bp amplification product. In addition, there is a second *Pst*I restriction site within the amplified fragment that serves as an internal control for digestion. The three possible genotypes are defined by three distinct banding patterns: AA (224- and 100-bp fragments), AC (224-, 158-, 100-, and 66-bp fragments), and CC (158-, 100- and 66-bp fragments) [10].

XPD exon 6: the sense primer P1(5′-TGG AGT GCT ATG GCA CGA TCT CT-3′) and the anti-sense primer P2 (5′-CCA TGG GCA TCA AAT TCC TGG GA-3′) amplified a 625-bp PCR product. PCR was performed in a total volume of 50 μL containing 300 ng of genomic DNA, 50 mmol/L KCl, 20 mmol/L Tris–HCl, 1.5 mmol/L $MgCl_2$, 200 μmol/L dNTP mix, 50 pmol/L of each primer (Invitrogen, UK), and 1 U Taq-polymerase (Invitrogen, UK). The amplification was carried out for 30 cycles under the following conditions: 1 min at 91°C for denaturation, 1 min at 52°C for primer annealing, and 1 min at 72°C for primer extension. An aliquot of the PCR product (about 10%) was digested with 3 U of *Tfi*I restriction enzyme (New England BioLabs, UK) at 65°C overnight (min. 3 h). Electrophoresis was performed on 3% agarose gel for 1.5 h at 150 V, and the DNA fragments were visualized with ethidium bromide.

A→C polymorphism change abolishes the *Tfi*I restriction site. The A but not the C allele has a *Tfi*I restriction site (G$^{\downarrow}$AA(T)TC) within the 625-bp amplified PCR product. The three possible genotypes are defined by three distinct banding patterns: CC (596- and 56-bp fragments), AC (596-, 482-, 114- and 56-bp fragments), and AA (482-, 114- and 56-bp fragments) [10].

Statistical Analysis

The odds ratio (OR) and its 95% confidence interval (95% CI) were used to analyze the frequencies of alleles and genotypes; the corresponding *P* values were calculated. A difference was determined significant for $P<0.05$. χ^2 test was used for evaluating differences of allele and genotype frequencies between cases and controls and to identify deviations from the Hardy–Weinberg proportion. All computations were undertaken using the statistical software ARCUS QUICKSTAT Biomedical Version 1.1 (Addison Wesley Longman Limited, UK) and STATISTICA Cz. Version 6.1 (StatSoft, Inc., VISA).

Table 3 XPD exon 6 genotypes and A, C allele frequencies in patients with breast cancer and controls

	XPD exon 6 genotypes			AC	Number
	AA, *n* (%)	AC, *n* (%)	CC, *n* (%)		
Controls	12 (10.6)	55 (48.7)	46 (40.7)	0.350 0.650	113
Breast cancer	32 (28)*	55 (48.3)	27 (23.7)	0.535** 0.465	114

**P*=0.003 (OR=3.03; 95% CI 1.35–7.07), differences of genotype distribution: Chi-square test. Heterozygous vs. Homozygous; ***P*< 0.0001 (OR=2.14; 95% CI 1.44–3.17), differences of allele frequencies: Chi-square test. Cases vs. controls

Results

In this study, we examined three SNPs in two different genes (exon 10 of XRCC1 and exons 6, 23 of XPD) in BC cases and controls. Tables 2, 3, 4 show the genotype distributions and allele frequencies for the SNPs in the BC cases and controls from Eastern region of Slovakia. For most SNPs the distribution between cases and controls did not differ. However, the genotype frequency for homozygotes AA in XPD exon 6 (Table 3) was significantly different between the BC group and the control group ($P=0.003$); the homozygotes AA appeared to be at risk of BC (OR=3.03; 95% CI 1.35–7.07). In the XPD exon 6 polymorphism a significant difference in the frequency of A allele was found between BC cases and the controls, too

Table 2 XRCC1 codon 399 (exon 10) genotypes and A, G allele frequencies in patients with breast cancer and controls

	XRCC1 codon 399 genotypes			AG	Number
	AA, *n* (%)	AG, *n* (%)	GG, *n* (%)		
Controls	53 (46.9)	43 (38.1)	17 (15)	0.660 0.340	113
Breast cancer	49* (43)	50 (43.9)	15 (13.1)	0.648** 0.352	114

**P*=0.39 (OR=0.8; 95% CI 0.43–1.42), differences of allele frequencies: Chi-square test. Cases vs. controls; ***P*=0.8 (OR=0.95; 95% CI 0.63–1.42), differences of genotype distribution: Chi-square test. Heterozygous vs. homozygous

Table 4 XPD exon 23 genotypes and A, C allele frequencies in patients with breast cancer and controls

	XPD exon 23 genotypes			AC	Number
	AA, *n* (%)	AC, *n* (%)	CC, *n* (%)		
Controls	46 (40.7)	50 (44.2)	17 (15.1)	0.628 0.372	113
Breast cancer	43 (37.7)*	53 (46.5)	18 (15.8)	0.604** 0.396	114

**P*=0.7 (OR=0.9; 95% CI 0.48–1.62), differences of genotype distribution: Chi-square test. Heterozygous vs. homozygous; ***P*= 0.6 (OR=0.90; 95% CI 0.61–1.34), differences of allele frequencies: Chi-square test. Cases vs. controls

($P<0.001$; OR=2.14; 95% CI 1.44–3.17). A test for the Hardy–Weinberg equilibrium (HWE) was performed for each SNP in the BC and control groups, and generally no population showed a systematic deviation from the HWE.

Discussion

The DNA repair is a system of defense designed to protect the integrity of the genome. Deficiencies in this system likely lead to the development of cancer. The epidemiology of DNA repair capacity and its effect on cancer susceptibility in humans is, therefore, an important area of investigation. Here, we investigated the relationship between DNA repair genes polymorphisms and the susceptibility to BC in women from the Eastern region of Slovakia. In the present study, the genotype distributions of the DNA repair genes XRCC1, XPD exon 6 and XPD exon 23 polymorphisms were found to be in HWE both in BC patients and healthy controls.

The polymorphism of the XRCC1 gene at exon 10 was recently predicted to be "possibly damaging" to the XRCC1 function based on the conservation of the sequences in mammalian orthologues [11]. However, studies investigating the relationship between cancer risk and XRCC1 polymorphisms have not consistently shown the evidence for an association. Inconsistent results have been found in lung cancer [12] and head and neck cancers [13]. No associations were observed in bladder [14] and prostate cancer [15]. Epidemiologic studies of the influence of the XRCC1 exon 10 polymorphism on the BC risk have provided some evidence for increased risk associated with the A allele among African Americans [5] and Asians [16]. Studies among Caucasians, however, have consistently found no association [17–20]. The lack of association in Caucasian populations was also confirmed by our results. We found no positive association between the polymorphism in codon 399 of XRCC1 gene and BC risk.

The determination of individual susceptibility to BC at DNA repair genes level is a complex problem. Because the etiology of BC is extremely complicated it is practically impossible to explain the susceptibility to breast cancer by means of only a single selected marker. Therefore, in the present work the hypothesis that genetic polymorphisms in the DNA repair mechanisms may influence susceptibility to BC was tested also through another polymorphic genes such as XPD exon 6 and XPD exon 23.

For the most commonly studied polymorphism of the XPD gene which affects the exon 23 no statistically significant associations have been found with respect to increased risk of bladder cancer, non-small cell lung cancer or melanoma [12, 21, 22]. However, there are some data on the association between BC risk and XPD polymorphism. In the present paper, no significant association with BC for the XPD exon 23 genotypes was found. This is in agreement with other studies on the XPD exon 23 polymorphism and BC risk including two Finnish [23, 24], one Danish [25], one German [26], two US studies in Caucasian women [27, 28] and one Brazilian study [29]. Interestingly, individuals with this polymorphism in the XPD gene may face an increased risk of BC from polycyclic aromatic hydrocarbons-DNA adducts and cigarette smoking [30].

The exon 6 polymorphism in the same gene does not result in an amino acid change. It is therefore unlikely that the enzymatic function of XPD could be affected by this variation. The polymorphism may influence the rate of translation by altering codon usage or reduce XPD protein levels through an effect on mRNA stability [10]. This study shows that the polymorphism in codon 156 of exon 6 influences the risk for the development of BC. While the *C*-allele seems to be protective, our results indicate that *A* allele in exon 6 is a risk factor for BC. Patients carrying two *A* alleles in exon 6 were at threefold higher risk of breast cancer than patients with two *C* alleles. The observed results are somewhat surprising because this variant is a silent polymorphism in exon 6 of the gene. The cases were significantly more likely than controls to be homozygous for the silent *AA* variant at codon 156 also in psoriatic patients with basal cell carcinomas or in patients with gliomas [10, 31].

This preliminary study suggests a possibility of an association between BC development and polymorphism in the *XPD* gene which makes this gene an important candidate for studies on the susceptibility to BC. However, since it is accepted that numerous genes (e.g., BRCA1/2) are involved in the development and progression of breast cancer, the gene–gene interactions needs to be considered in further studies.

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References

1. Parkin DM, Bray F, Ferlay J et al (2005) Global cancer statistics. CA Cancer J Clin 55:74–108
2. Mohrenweiser HW, Jones IM (1998) 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 400:15–24
3. Shen M, Jones I, Mohrenweiser H (1998) Nonconservative amino acid substitutions variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res 58:604–608

4. Moullan N, Cox DG, Angele S et al (2003) Polymorphisms in the DNA repair gene XRCC1, breast cancer risk, and response to radiotherapy. Cancer Epidemiol Biomarkers Prev 12:1168–1174
5. Duell EJ, Millikan RC, Pittman GS et al (2001) Polymorphisms in the DNA repair gene XRCC1 and breast cancer. Cancer Epidemiol Biomarkers Prev 10:217–222
6. Benhamou S, Sarasin A (2002) ERCC2/XPD gene polymorphisms and cancer risk. Mutagenesis 6:463–469
7. Stary A, Sarasin A (2001) ERCC2 (Excision repair cross-complementing rodent repair deficiency, complementation group 2). Atlas Genet Cytogenet Oncol Haematol. http://www.infobiogen.fr/services/chromcancer/Genes/XPDID297.html
8. Miller S, Dykes D, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215
9. Lunn RM, Langlois RG, Hsieh LL et al (1999) XRCC1 polymorphism: effect on aflatoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res 59:2557–2561
10. Dybdahl M, Vogel U, Frentz G et al (1999) Polymorphisms in the DNA repair gene XPD: Correlations with risk and age at onset of basal cell carcinoma. Cancer Epidemiol Biomarkers Prev 8:77–81
11. Savas S, Kim DY, Ahmad MF et al (2004) Identifying functional genetic variants in DNA repair pathway using protein conservation analysis. Cancer Epidemiol Biomarkers Prev 13:801–807
12. Butkiewicz D, Rusin M, Enewold L et al (2001) Genetic polymorphisms in DNA repair genes and risk of lung cancer. Carcinogenesis 22:593–597
13. Olshan AF, Watson MA, Weissler MC et al (2002) XRCC1 polymorphisms and head and neck cancer. Cancer Lett 178:181–186
14. Stern MC, Umbach DM, van Gils CH et al (2001) DNA repair gene XRCC1 polymorphisms, smoking, and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 10:125–131
15. van Gils CH, Bostick RM, Stern MC et al (2002) Differences in base excision repair capacity may modulate the effect of dietary antioxidant intake on prostate cancer risk: an example of polymorphisms in the XRCC1 gene. Cancer Epidemiol Biomarkers Prev 11:1279–1284
16. Chacko P, Rajan B, Joseph T et al (2005) Polymorphisms in DNA repair gene XRCC1 and increased genetic susceptibility to breast cancer. Breast Cancer Res Treat 89:15–21
17. Smith TR, Miller MS, Lohman K et al (2003) Polymorphisms of XRCC1 and XRCC3 genes and susceptibility to breast cancer. Cancer Lett 190:183–190
18. Brewster AM, Jorgensen TJ, Ruczinski I et al (2006) Polymorphisms of the DNA repair genes XPD (Lys751Gln) and XRCC1 (Arg399Gln and Arg194Trp): relationship to breast cancer risk and familial predisposition to breast cancer. Breast Cancer Res Treat 95:73–80
19. Thyagarajan B, Anderson KE, Folsom AR et al (2006) No association between XRCC1 and XRCC3 gene polymorphisms and breast cancer risk: Iowa Women's Health Study. Cancer Detect Prev 30:313–321
20. Zhang Y, Newcomb PA, Egan KM et al (2006) Genetic polymorphisms in base-excision repair pathway genes and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 15:353–358
21. Matullo G, Guarrera S, Carturan S et al (2001) DNA repair gene polymorphisms, bulky DNA adducts in white blood cells and bladder cancer in a case-control study. Int J Cancer 92:562–567
22. Winsey SL, Haldar NA, Marsh HP et al (2000) A variant within the DNA repair gene XRCC3 is associated with the development of melanoma skin cancer. Cancer Res 60:5612–5616
23. Forsti A, Angelini S, Festa F et al (2004) Single nucleotide polymorphisms in breast cancer. Oncol Rep 11:917–922
24. Metsola K, Kataja V, Sillanpaa P et al (2005) XRCC1 and XPD genetic polymorphisms, smoking and breast cancer risk in a Finnish case-control study. Breast Cancer Res 6:R987–R997
25. Nexo BA, Vogel U, Olsen A et al (2003) A specific haplotype of single nucleotide polymorphisms on chromosome 19q13.2-3 encompassing the gene RAI is indicative of post-menopausal breast cancer before age 55. Carcinogenesis 24:899–904
26. Justenhoven C, Hamann U, Pesch B et al (2004) ERCC2 genotypes and a corresponding haplotype are linked with breast cancer risk in a German population. Cancer Epidemiol Biomarkers Prev 13:2059–2064
27. Tang D, Cho S, Rundle A et al (2002) Polymorphisms in the DNA repair enzyme XPD are associated with increased levels of PAH-DNA adducts in a case-control study of breast cancer. Breast Cancer Res Treat 75:159–166
28. Shi Q, Wang LE, Bondy ML et al (2004) Reduced DNA repair of benzo(a)pyrene diol epoxide-induced adducts and common XPD polymorphisms in breast cancer patients. Carcinogenesis 25:1695–1700
29. Dufloth RM, Costa S, Schmitt F et al (2005) DNA repair gene polymorphisms and susceptibility to familial breast cancer in a group of patients from Campinas, Brazil. Genet Mol Res 4:771–782
30. Terry MB, Gammon MD, Zhang FF et al (2004) Polymorphism in the DNA repair gene XPD, polycyclic aromatic hydrocarbon-DNA adducts, cigarette smoking, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 13:2053–2058
31. Caggana M, Kilgallen J, Conroy JM et al (2001) Associations between ERCC2 polymorphisms and gliomas. Cancer Epidemiol Biomarkers Prev 10:355–360