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SUSCEPTIBILITY TO BREAST CANCER AND INTRON 3 INS/DEL GENETIC POLYMORPHISM OF DNA DOUBLE-STRAND BREAK REPAIR GENE *XRCC4*

PODLOŽNOST RAKU DOJKE I GENETSKI INS/DEL POLIMORFIZAM U INTRONU 3 GENA XRCC4 ZA REPARACIJU DVOLANČANIH PREKIDA DNK

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Summary

Background: Since genetic variations in X-ray cross-complementing group 4 (*XRCC4*; OMIM: 194363) repair gene might be associated with a reduction in cellular DNA repair capacity, it is hypothesized that *XRCC4* Ins/Del (I/D) polymorphism (in intron 3 of the gene; rs28360071) may be a risk factor for breast cancer. Therefore, the present case-control study was carried out.
Methods: The present case-control study included 407 females with breast cancer and a total of 394 healthy females from the general population matched with patients according to age. Genotypic analysis for the *XRCC4* I/D polymorphism was performed by PCR. In order to investigate the effect of *XRCC4* I/D polymorphism on age at diagnosis of breast cancer, the Kaplan–Meier survival analysis and the Cox proportional hazards regression model were used.
Results: Based on the present case-control study, the ID (OR=0.95, 95% CI: 0.69–1.31, P=0.781) and DD (OR=1.24, 95% CI: 0.84–1.83, P=0.274) genotypes were not associated with breast cancer risk compared with the II genotype. Based on the Cox regression model, there was significant association between genotypes of I/D polymorphism and age at diagnosis of breast cancer (ID+DD vs II; HR=0.79, 95% CI: 0.64–0.98, P=0.036).
Conclusion: Although there was no significant association between *XRCC4* I/D polymorphism and risk of breast cancer, patients having the II genotype have lower age at diagnosis in comparison with patients having ID+DD genotypes.

Keywords: age at diagnosis, breast cancer, polymorphism, susceptibility, XRCC4

Kratak sadržaj

Uvod: Pošto su genetske varijacije u reparatornom genu X-ray cross-complementing group 4 (XRCC4; OMIM: 194363) možda povezane sa smanjenjem ćelijskog kapaciteta za reparaciju DNK, postavljena je hipoteza da Ins/Del (I/D) polimorfizam XRCC4 (u intronu 3 tog gena; rs28360071) može biti faktor rizika za rak dojke. Iz tog razloga, sprovedeno je ovo ispitivanje.
Metode: Ova anamnestička studija obuhvatila je 407 žena sa kancerom dojke i ukupno 394 zdrave žene iz opšte populacije uparene sa pacijentkinjama prema starosti. Genotipska analiza za I/D polimorfizam XRCC4 obavljena je pomoću PCR. Za istraživanje uticaja I/D polimorfizma XRCC4 na starost prilikom dijagnostikovanja kancera dojke korišćeni su Kaplan–Meierova analiza preživljavanja i Coxov regresioni model proporcionalnih rizika.
Rezultati: Na osnovu ove anamnestičke studije, ID (OR=0,95, 95% CI: 0,60–1,31, P=0,781) i DD (OR=1,24, 95% CI: 0,84–1,83, p=0,274) genotipi nisu bili povezani sa rizikom od kancera dojke u poređenju sa II genotipom. Na osnovu Coxovog regresionog modela, postojala je značajna povezanost između genotipova I/D polimorfizma i starosti prilikom dijagnostikovanja kancera dojke (ID+DD vs II; HR=0,79, 95% CI: 0,64–0,98, P=0,036).
Zaključak: Iako nije bilo značajne povezanosti između I/D polimorfizma XRCC4 i rizika od kancera dojke, pacijentkinje sa II genotipom imaju manje vrednosti za starost prilikom dijagnoze u poređenju s pacijentkinjama sa II+DD genotipovima.

Ključne reči: starost prilikom dijagnoze, kancer dojke, polimorfizam, podložnost, XRCC4

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Introduction

There are two main pathways for DNA double-strand breaks (DSBs) repair which are named homologous recombination and non-homologous end-joining (NHEJ). The NHEJ is the major pathway to repair DNA DSBs in mammalian species (1, 2). In the NHEJ pathway, a relatively small number of essential repair proteins mediate the DSB repair, including X-ray cross-complementing group 4 (XRCC4; OMIM: 194363) (1, 2). The *XRCC4* encodes a ubiquitously expressed product (2).

It has been reported that inactivation of the *XRCC4* gene in mice via gene-targeted mutation results in *XRCC4* deficiency in primary murine cells that causes growth defects, premature senescence, IR sensitivity, inability to support V(D)J recombination, late embryonic lethality accompanied by defective lymphogenesis and defective neurogenesis manifested by extensive apoptotic death of newly generated post-mitotic neuronal cells (3).

Previously, it has been suggested that polymorphism of DNA repair genes is associated with increased risk of several types of cancers (4–17). Several genetic polymorphisms in the *XRCC4* gene have been described for lung, gastric, prostate, breast, bladder, oral and brain cancers, multiple myeloma and childhood leukemia (see http: //www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?geneId=7518; 4–9, 18–22). One of the described polymorphisms is an Insertion/Deletion *XRCC4* polymorphism (I/D; rs28360071). This polymorphism occurred by an insertion/or deletion of a 30 bp sequence (GAT GAG GAA ACT AAC TCT CAG TGG TGT TTA) in intron 3 on the *XRCC4* gene (http: //www.ncbi.nlm.nih.gov/snp/?term=rs 28360071). Several association studies were performed on the *XRCC4* I/D polymorphism and susceptibility to different cancers (4–11, 23–26). There are, however, two studies indicating that this polymorphism is significantly associated with altered risk of prostate and oral cancers (6, 11).

A meta-analysis of five case-control studies demonstrates that the rs2075685 (G>T) and rs6869366 (G>T) polymorphisms of *XRCC4* might increase the risk of breast cancer (18). However, there is no published study investigating the association between the I/D genetic polymorphism of *XRCC4* and susceptibility to breast cancer. Since genetic variations in *XRCC4* repair gene might be associated with a reduction in cellular DNA repair capacity (21), it is essential to carry out the present studies on the association between I/D *XRCC4* polymorphism and breast cancer risk.

Materials and Methods

Participants

The present population-based case–control study used participants from our previous study (27). The study group consisted of 407 females with breast cancer that were recruited from the chemotherapy department of Nemazi hospital in Shiraz (south-west of Iran). A total of 394 healthy females from the general population were matched with patients according to age and served as a control group. The mean age of the patients and the controls was 43.9±8.8 years and 45.2±10.7 years, respectively.

Because the Iranian population is one of the most heterogeneous populations (28, 29), we have selected our patients and controls from Persian (Caucasian) Muslims living in Shiraz. Informed consent was obtained from each subject before the study. This study was approved by the local ethics committee. The present study is more than sufficiently powered with an $N=801$ to detect a small-medium effect in allelic frequency between the two groups. Using the GPOWER (www.psycho.uni-duesseldorf.de/aap/projects/gpower) software (version 2.0), to detect a real difference in allelic frequency with a power of 0.95 ($\alpha=0.01$), $df=1$, Lambda=17.84, and an effect size of 0.2, a minimum sample of 446 would be necessary.

Genotyping

Blood samples were obtained from patient and control groups. Immediately after collection, whole blood was stored at –20 °C until use. Genomic DNA for PCR was isolated from whole blood using the thawed blood samples by standard procedure as described previously (30). Genotypic analysis for the *XRCC4* I/D polymorphism was determined as described previously (4). Any sample with an ambiguous result due to low yield was retested and a random selection of 15% of all samples was repeated. No discrepancies were discovered upon replicate testing.

Statistical analysis

For the control group of the study, the observed frequencies of the *XRCC4* genotypes were assessed for Hardy-Weinberg equilibrium using the χ^2 statistic. The associations between the genotypes of *XRCC4* I/D polymorphism and risk of breast cancer were assessed by calculating odds ratios (ORs) and 95% confidence intervals (CIs). To determine the effect of I/D *XRCC4* polymorphism on age at diagnosis of breast cancer, the Kaplan-Meier survival analysis and the Cox proportional hazards regression model were used. In the analysis, breast cancer was defined as event and age at diagnosis was included in the analysis as time period to event.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) (version 11.5). A probability of $P<0.05$ was considered statistically significant. All P values were two-tailed.

Results and Discussion

Table I shows the genotypes of *XRCC4* I/D polymorphism in breast cancer cases and healthy controls. The genotypic frequencies of the control subjects did not show significant deviation from the Hardy-Weinberg equilibrium (χ^2=0.03, df=1, P=0.872). *Table I* also shows the association between the *XRCC4* I/D polymorphism and breast cancer risk. The ID (OR=0.95, 95% CI: 0.69–1.31, P=0.781) and DD (OR=1.24, 95% CI: 0.84–1.83, P=0.274) genotypes were not associated with breast cancer risk compared to the II genotype. Also, there is no linear trend for the presence of 0, 1, and 2 D allele and the risk of breast cancer (χ^2=0.81, P=0.367). The same results were revealed when the participants were stratified by the menopausal status (*Table I*). It should be noted that there was no significant association between the study polymorphism and risk of breast cancer in both smoker and non-smoker participants (data not shown).

To the best of our knowledge, this is the first report of an association between a potentially functional I/D polymorphism of the *XRCC4* gene and the risk of developing breast cancer, with a reasonable sample size (a total of 801 participants). In the present population-based case-control study, we found no significant association between the risk of breast cancer and *XRCC4* I/D polymorphism (*Table I*). Several studies have investigated the association between *XRCC4* I/D polymorphism and cancers (4–11, 23–26). The results were not consistent. Considering that the rs2075685 (G>T) and rs6869366 (G>T) polymorphisms of *XRCC4* are associated with the risk of breast cancer (18), it might be concluded that although the *XRCC4* is involved in breast cancer susceptibility, the I/D polymorphism of *XRCC4* is not involved in breast carcinogenesis.

The Kaplan-Meier survival analysis revealed that the genotypes of *XRCC4* I/D polymorphism (log rank statistic=3.59, df=1, P=0.058) and smoking habit were associated with age at diagnosis of breast cancer (log rank statistic=5.35, df=1, P=0.021). In the Cox proportional hazards regression model, smoking status and the genotypes of *XRCC4* I/D polymorphism were treated as categorical variables in the model. Hazard ratios (HR) and 95% confidence intervals (CI) for categorical variables were estimated. Based on the Cox regression model, there was a statistically significant association between the I/D poly-

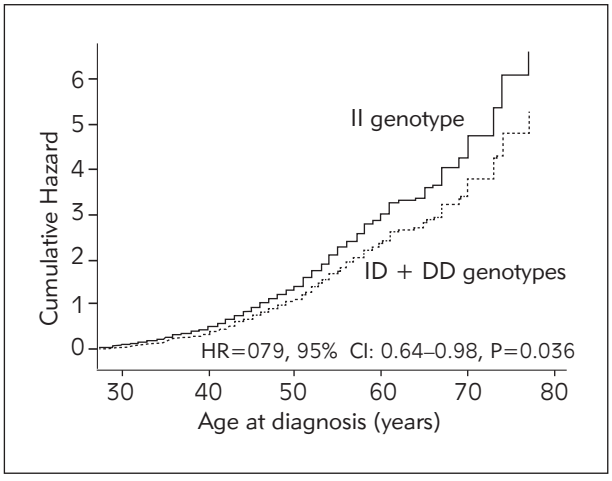

Figure 1 Association between Insertion/Deletion *XRCC4* polymorphism and age at diagnosis of breast cancer.

Table I Genotypic distribution of the Insertion/Deletion *XRCC4* polymorphism among cases and controls.

Subjects/Genotypes	Controls	Cases	OR	95% CI	P-value
All subjects					
II	127	128	1.0	–	–
ID	192	185	0.95	0.69–1.31	0.781
DD	75	94	1.24	0.84–1.83	0.274
ID+DD	267	279	1.03	0.77–1.39	0.812
Premenopausal					
II	92	85	1.0	–	–
ID	143	104	0.78	0.53–1.16	0.227
DD	54	57	1.14	0.71–1.83	0.582
ID+DD	197	161	0.88	0.61–1.26	0.505
Postmenopausal					
II	32	30	1.0	–	–
ID	44	49	1.18	0.62–2.26	0.600
DD	20	21	1.12	0.50–2.46	0.778
ID+DD	64	70	1.16	0.63–2.13	0.616

Note: There is no linear trend for the presence of 0, 1 and 2 D allele (χ^2=0.81, P=0.367) and risk of breast cancer.

morphism and age at diagnosis of breast cancer (HR=0.79, 95% CI: 0.64–0.98, P=0.036). This means that patients having the II genotype have lower age at diagnosis in comparison with patients having ID+DD genotypes (*Figure 1*).

It is suggested that susceptibility to breast cancer and age at diagnosis of breast cancer are different multifactorial traits. There were other reports that indicated that specific polymorphisms may be associated with risk of a disease or the age at which the disease is diagnosed (12, 13).

The major limitation of the present study is that we studied only the I/D polymorphism in intron 3 of the *XRCC4* gene. There are several other single nucleotide polymorphisms for *XRCC4* in humans (please see http: //www.ncbi.nlm.nih.gov/ projects/ SNP/snp_ref.cgi?geneId=7518), which were not studied in the present work. Simultaneous study of these polymorphisms (including the haplotype analysis) should be further researched.

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Conflict of interest statement

The authors stated that have no conflicts of interest regarding the publication of this article.

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