

## DNA REPAIR GENE XRCC3 241MET VARIANT AND BREAST CANCER SUSCEPTIBILITY OF AZERI POPULATION IN IRANIAN

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DNA-repair systems are essential for repairing damage that occurs when there is recombination between homologous chromosomes. The gene XRCC3 (X-ray cross complementing group 3) encodes a member of the RecA/Rad51-related protein family that participates in homologous recombination to maintain chromosome stability and repair DNA damage. The Thr241Met (XRCC3-18067C>T, rs861539) substitution, a C to T transition at codon 241 in exon 7, thus plays critical roles in cancer development. The aim of this study was association between XRCC3 Thr241Met polymorphism and risk of sporadic breast cancer in Azari population. We analysed DNA samples from 100 sporadic breast cancer patients and 100 healthy women, for XRCC3 Thr241Met polymorphism using PCR-RFLP. Genotype specific risks were tested using chi<sup>2</sup>-test with 95% confident intervals. Frequency of Thr/Thr at codon 241 was 69% in controls and 70% in patients, Thr/Met frequency was 22% in controls and 13% in patients, the Met/Met genotype was 9% in controls and 17% in patients. No correlation between the genotype and allele distribution and increased susceptibility for breast cancer. Our results suggested that in premenopausal women, breast cancer risk is not significantly associated with rs861539 in Azari population.

*Key words:* Breast cancer, Polymorphism, XRCC3

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## INTRODUCTION

DNA repair systems play main roles in protecting against mutations and are essential for maintaining the integrity of the genome. Certain most genetic variants within the genes involved in DNA damage responses may contribute to the development of some cancers skin, bladder, breast and lung (DUARTE *et al.*, 2005). Because reduced DNA repair capacity may lead to genetic instability and cell cytotoxic, carcinogenesis. Genes involved in DNA repair have been proposed as candidate cancer susceptibility genes (WOOD *et al.*, 2001). Some proteins having established roles in DNA repair pathways including nucleotide excision repair (NER), base excision repair (BER), double-strand break repair (DSBR) and mismatch repair (MMR) (WOOD *et al.*, 2001; YU *et al.*, 1999). XRCC3(X-ray repair complementing defective repair in Chinese hamster cells 3 is a member of the RecA/Rad51- related protein family involved in the repair of double-strand breaks (DSB) and interstrand crosslinks repair (ECONOMOPOULOS *et al.*, 2010).The XRCC3 gene is located on the long (q) arm of chromosome 14 at position 32.3 (CUSTODIO *et al.*, 2012).The main effective variant in the XRCC3 gene, involving a C to T (rs861539) substitution designated as XRCC3 results in a threonine (Thr) to methionine (Met) amino acid change at codon 241 in exon 7(C>T). This variant could affect phosphorylation site and thus affect repair function or interaction with other proteins involved in repair (DUARTE *et al.*, 2005).Thr241Met SNP in XRCC3 gene is the reason for the defect in its function and were detected in breast cancer and associate with clinical phenotypes. XRCC3 241Met variant change the amino acids coded from one with a neutral hydrophilic hydroxyl group (Thr) to a hydrophobic one with a methyl sulfur group (Met ) (LEE *et al.*, 2005). In our case-control study was conducted to examine the genotype distribution of rs861539 and to search for an association between risk of sporadic breast cancer and XRCC3 SNP, using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach.

## MATERIALS AND METHODS

### *Samples*

In the current study, blood samples collected from 100 patients with sporadic breast cancer and were in premenopausal status in northwest of Iran .Hospital. Blood samples for control obtained from 100 women without any cancer. The mean age for all breast cancer patients and Control women were 39.32.

### *Genotyping*

DNA was extracted from blood by salting out method. Genotyping was performed using the polymerase chain reaction (PCR) restriction fragment length polymorphism method, with the digested PCR products applied to electrophoresis on agarose gels. The following primers were used: forward primer 5'GGTCGAGTGACAGTCCAAAC3' and reverse primer 5'TGCAACGGCTGAGGGTCTT3'. PCR was obtained in a 25µl reaction mixture containing 100 ng of genomic DNA template, 1X PCR buffer, 200 mM dNTP mix., 10 Pmol each of the forward and reverse primers, and 1 U of Taq DNA polymerase .The reaction conditions used were initial denaturation at 95\_C for 2 minutes, followed by 31 step-cycles of denaturation at 95\_C for 30 seconds, annealing at 60\_C for 45 seconds, and extension at 72\_C for 30 seconds followed by a terminal extension time of

10 minutes. Ten microliters of PCR product were digested with *NlaIII* restriction enzyme (Thermo Fisher Scientific, Inc., Marietta, OH, USA) for 12 hours at 60 °C. The digestion products were then resolved on a 8% polyacrylamide gel. The wild homozygote (T/T) allele was cleaved by *NlaIII*, yielding two small fragments 239 and 313bp. Heterozygotes (T/M) contained four bands and mutant homozygote (M/M) corresponding to 105, 208 and 239bp.

#### Statistical analysis

The calculation of allele frequencies of the XRCC3 polymorphism was based on the observed allele and genotype distributions among controls and patients. The differences of the allele and genotype frequencies were analyzed using the Chi-square test or with 95% confidence intervals was performed using SPSS v.16 software. Statistical significance was set at  $P < 0.05$ .

## RESULTS

No statistically significant differences were observed in genotype frequencies ( $p=0.08$ ) and allele frequencies ( $p=0.4$ ) of XRCC3 Thr241Met polymorphism between female patients and controls. The proportion of Thr/Thr, Thr/Met and Met/Met genotypes in breast cancer patients was found to be 70%, 13% and 17% respectively, as compared to 69%, 22% and 9% in the control individuals. The relative frequency of each allele was 0.76 for Thr and 0.24 for Met in patients with breast cancer, and 0.80 for Thr and 0.20 for Met in normal controls.

Table 1. Allele and genotype frequencies in breast cancer patients and in healthy women

Polymorphisms	Cases n (%)	Controls n (%)	OR(95%CI)	P
<b>SNP XRCC3</b>				
<b>Genotypes</b>				
Thr/Thr	70	69		Ref
Thr/Met	13	22	1.71(0.80-3.67)	0.18
Met/Met	17	9	0.53(0.22-1.28)	0.11
<b>Alleles</b>				
Thr	153(76.5%)	160(80%)	Ref	Ref
Met	47(23.5%)	81(20%)	0.82(0.51-1.32)	0.42

## DISCUSSION

Breast cancer is the most frequent invasive malignancy affecting women all over the world and is considered to be the third most common malignancy in the world with more than 1.3 million women diagnosed with breast cancer each year (VIJAYARAMAN *et al.*, 2012; TIKHOMIROVA *et al.*, 2007). Various risk factors are associated with human breast cancer development. Genetic factors are important in

breast cancer. Environment and genetic factors might lead to cancer formation (XU *et al.*, 2010; OSTASHKIN *et al.*, 2007). SNPs have important roles in human genetics and medicine and have been widely used in genetic association studies of various cancer (CURRAN *et al.*, 2001; MILLER *et al.*, 2001; LIN *et al.*, 2003; TAMURA *et al.*, 2003; YAMADA *et al.*, 2003; HIRAI *et al.*, 2005). Single nucleotide polymorphisms in DNA repair pathway lead to cellular genetic instability which in turn may lead to cancer development (LEE *et al.*, 2005). Double strand breaks (DSB) in repair genes may play a role in genome integrity. Homologous recombination (HR) and nonhomologous end joining (NHEJ) have main role in DSB repair (ROMANOWICZ-MAKOWSKA., 2012). The XRCC3 protein is involved in the homologous recombination repair (HR) pathway, responsible for DNA double-strand break repair (LEE *et al.*, 2005). The XRCC3 Thr241Met polymorphism has been investigated in many types of cancer and the findings have been varied. The study of these genotypic variations in DNA repair functions and their association with cancer may help to elucidate cancer etiology. The XRCC3, Thr 241 Met variation is evolutionarily conserved and functional data supports that it could be a risk allele for breast cancer (LEE *et al.*, 2005). A meta-analysis of 15 case-control studies hypothesized that the XRCC3 Thr241Met polymorphism may modify the risk of colorectal cancer, particularly in Asian population (WANG *et al.*, 2013). Some authors have reported the role of rs861539 or XRCC3 T241M polymorphism with an increased risk of breast cancer (SILVA *et al.*, 2010; ZHANG *et al.*, 2005; THYAGARAJAN *et al.*, 2006; HAN *et al.*, 2004; WEBB *et al.*, 2005; GARCÍA-CLOSAS *et al.*, 2006), however the results remained controversial. A meta-analysis of 17 published case-control studies indicated that the XRCC3 241Met allele was not associated with the risk of lung cancer (FAN *et al.*, 2013). LOIZIDOU *et al.* studied the XRCC3 Thr241Met polymorphism in samples of breast cancer and concluded that the genotype Thr/Met and homozygous Met/Met do not associated with increased risk for developing this cancer (LOIZIDOU *et al.*, 2008). In the Polish population were observed slightly increase risk between the XRCC3 Thr241Met polymorphism and breast cancer (KRUPA *et al.*, 2009). A meta-analysis of case-control studies (including 3,191 cases and 5,090 controls) reported that rs861539 may act as a head and neck cancer risk factor among all subjects (LI *et al.*, 2012). A meta-analysis of 36 case-control studies suggests that the T241M polymorphism confers a weakly increased breast cancer (MAO *et al.*, 2014). In our study we investigated whether XRCC3, Met 241 Met genotype were associated with the risk of sporadic breast cancer. Interestingly there was not any noticeable difference in distribution of allele frequencies of rs861539 in both case and control groups. We didn't find a significant positive association between the XRCC3 codon 241 Met allele and premenopausal breast cancer in this population (Table 1). Allele frequencies at the XRCC3 gene polymorphism in our study were inconsistent with in Russians and Tatars from the Republic of Bashkortostan (Kochetova *et al.*, 2013). The most recent meta-analysis on the field has reported that the Met allele is associated with risk of breast cancer in Asian and Caucasian population (ECONOMOPOULOS *et al.*, 2010). Finally, it is postulated that this polymorphism can't be used as a predictive factor for breast cancer in the Azari female population.

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**MUTANT GENA XRCC3 241Met KOJI KONTROLIŠE POPRAVKU DNK I OSETLJIVOST KANCERA DOJKE KOD AZERI POPULACIJE U IRANU**

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**Izvod**

DNK – reper sistemi su ključni za popravku oštećenja kada dolazi do rekombinacije homologih hromozoma. Gen XRCC3 (X-ray cross complementing group 3) kodira jednog člana RecA/Rad51 - zavisne porodice protein koji učestvuje u homologim rekombinacijama u cilju održanja stabilnosti hromozoma i popravku oštećenja. Thr241Met (XRCC3-18067C>T, rs861539), substitucije odnosno tranzicija C u T u kodonu 241 u egzonu 7 koja ima kritičnu ulogu u razvoju kancera. Cilj ovih ispitivanja je bila asocijacija između XRCC3 Thr241Met polimorfizma i rizik sporadičnog kancera dojke u Azari populaciji. Vršena su ispitivanja polimorfizma XRCC3 Thr241Met u uzorcima DNK 100 sporadičnih pacijenata i 100 zdravih žena, metodom PCR-RFLP. Naši rezultati ukazuju da kod žena u fazi pre menopauze rizik pojave kancera dojke nije značajno povezan sa rs861539 u Azari populaciji

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