

Genetic association of *XRCC5* gene polymorphisms with breast cancer among Jordanian women

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Purpose: Breast cancer (BC) is a complex disease that is governed by several different environmental and inherited factors. There are many genes have been linked with BC development by screening specific genetic variants within these genes. In this study, we aim to investigate the correlation between Variable Number Tandem Repeat (VNTR) in *XRCC5* gene and BC.

Materials and methods: Polymerase Chain Reaction (PCR) and Gel electrophoresis were used to genotype the *XRCC5* gene polymorphism in 200 cases with breast cancer and 200 healthy individuals. All participants were Jordanian women from Arab descents. Clinical and pathological characteristics for BC patients were summarized and categorized according to their medical records.

Results: In this study, we found a strong correlation between the VNTR polymorphism in the *XRCC5* gene and BC risk (P -value<0.0001). Remarkably, three different genotypes (2R\2R, 3R\2R and 3R\3R) showed significant association with BC (P -value<0.0001). This study also reported a significant difference in the distribution of allele frequencies between BC patients and healthy individuals (3R; P -value<0.0001 and 2R; P -value<0.001). However, we propose that VNTR of *XRCC5* gene did not interfere with BC prognosis.

Conclusion: We speculate that the VNTR of *XRCC5* gene may influence BC development. More investigations are needed in this regard to clarify the underlying role of the *XRCC5* genetic variant in tumorigenesis including BC development.

Keywords: breast, cancer, *XRCC5*, genetic polymorphism, prognosis

Background

While breast cancer (BC) can occur in men, it is much more frequent in women, and it is considered the most prevalent and fatal type of cancer affecting women.¹⁻³

In Jordan, BC is the most common female cancer accounting for 38.2% of all cancers BC according to GLOBOCAN reports. Survival rates have increased in recent years due to wider awareness of preventative measures as well as enhanced therapeutic protocols.⁴ Although the precise cause of BC is still not fully clear, the main risk factors for the disease have been established.^{5,6} In the past decade, the spotlight of BC research has shifted to the role of inherited risk factors, such as family history and genetic mutations, in the disease's progression.⁷⁻⁹ It has been reported that the occurrence of certain genetic polymorphisms in critical house-keeping genes contributes to the onset of cancer.^{10,11}

The variable number tandem repeat (VNTR) polymorphism in the X-ray repair cross-complementing protein 5 (*XRCC5*) gene has been linked to the development of cancer in Iranians and Caucasians.¹²⁻¹⁴ More specifically, this 160-bp VNTR is located

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in the *XRCC5* promoter region and involves a 21-bp repetitive unit (TGC GCATGCTCGGCGGGAATC).^{15,16} The *XRCC5* gene is located on chromosome 2 and encodes the DNA repair protein Ku80.¹⁷ Four different VNTR alleles have been identified: 0R, 1R, 2R and 3R.¹⁸

A limited number of studies have investigated the relationship between BC and the *XRCC5* VNTR polymorphism. Rajaei et al,¹⁸ reported that the 0R/0R genotype led to an increased BC risk compared with the 1R/1R genotype, while Cui et al,¹⁴ found that the VNTR in the *XRCC5* promoter was associated with an altered BC risk. The primary aim of the current study is to investigate the impact of the *XRCC5* promoter VNTR in BC risk as well as its prognosis in the Jordanian population of Arab descent.

Methods

Study population

Ethical approval (Code number: 32/104/2017) from the Intuitional Review Board (IRB) at Jordan University of Science and Technology (JUST) along with written informed consent was obtained for this study and from all its participants, respectively. This study was also conducted in accordance with the Declaration of Helsinki. In total, the 400 samples were utilized for genomic extraction and genotyping: 200 samples from Jordanian BC patients were collected from the Jordanian Royal Medical Services (JRMS) hospital, in addition to 200 samples from randomly selected healthy Jordanian women were recruited from the blood bank at JRMS. Both the patients and the controls were age- and gender-matched and came from the same ethnic background (Arab). In addition, demographic and clinical data of BC samples were extracted from the medical records of DE-identified Arab BC patients.

Genomic extraction and genotyping *XRCC5* promoter VNTR polymorphisms

Five milliliters of blood was collected from each participant and DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega Corp., Madison, WI, USA) according to the manufacturer's instructions. DNA quantity and quality were then assessed using the NanoDrop™ (Thermo Scientific, USA). The polymerase chain reaction (PCR) technique coupled with agarose gel electrophoresis was employed to genotype the VNTR in the *XRCC5* promoter of each participant. Briefly, 2 µL each of sense (5'AGGCGGCTCAAACACCACAC3') and antisense (5'CAAGCGGCAGATAGCGAAAG3')¹⁴ was

added to 12.5 µL of ready-to-use master mix, 10 µL of distilled deionized water and 50 ng of DNA. The samples were loaded into a thermal cycler set to the following conditions: initial denaturation at 95° C for 7 mins, 35 cycles of denaturation (95° C for 30 s), annealing (62° C for 30 s), and extension (72° C for 45 s), and a final extension at 72° C for 7 mins. The amplicon was separated using 2% agarose gel stained with ethidium bromide (0.07 µg/mL) and placed in TBE buffer. Afterwards, the bands were visualized and documented by a gel image system. The size of the DNA bands was determined through comparison with a 100 bp ladder.

Statistical analysis

Genotypic and allelic frequencies were calculated, and Pearson's chi-squared test was applied to compare between cases and controls by calculating the *P*-value.¹⁹ The Pearson χ^2 test was also used to evaluate the deviation of the Hardy–Weinberg equilibrium (HWE). The HWE equation ($p^2+2pq+q^2+2qr+r^2+2ps+2pr+2qs+2sr+s^2=1$) was used to calculate the estimated multiple allelic (0R, 1R, 2R and 3R) and genotypic (0R\0R, 1R\0R, 1R\1R, 1R\2R, 2R\0R, 2R\2R, 1R\3R, 3R\2R and 3R\3R) frequencies. In this study, Pearson's Chi-squared and ANOVA tests were also used to perform the genotype-phenotype analyses. A *P*-value of less than 0.05 was considered statistically significant. The Statistical Package for the Social Sciences (SPSS) version 21.0 was also used to perform all analyses.

Results

VNTR frequency distributions in the *XRCC5* promoter among breast cancer (BC) patients and controls

After performing the HWE test for the polymorphism in both cases and controls, it was in accordance with the HWE criteria. Our results involved the following four VNTR alleles: 0R, 1R, 2R and 3R. Each allele possessed a specific molecular weight (Figure 1). Nine different genotypes can result from these alleles. Table 1 summarizes the distribution of these different genotypes and alleles among BC patients and controls.

As can be seen from Table 1, 35.5% of healthy individuals possessed the 2R\2R genotype, while the 1R\1R genotype was found in 25% of healthy subjects. In contrast, both the 3R\3R and 0R\0R genotypes accounted for only 1% of healthy subjects. According to our findings, the 2R\2R

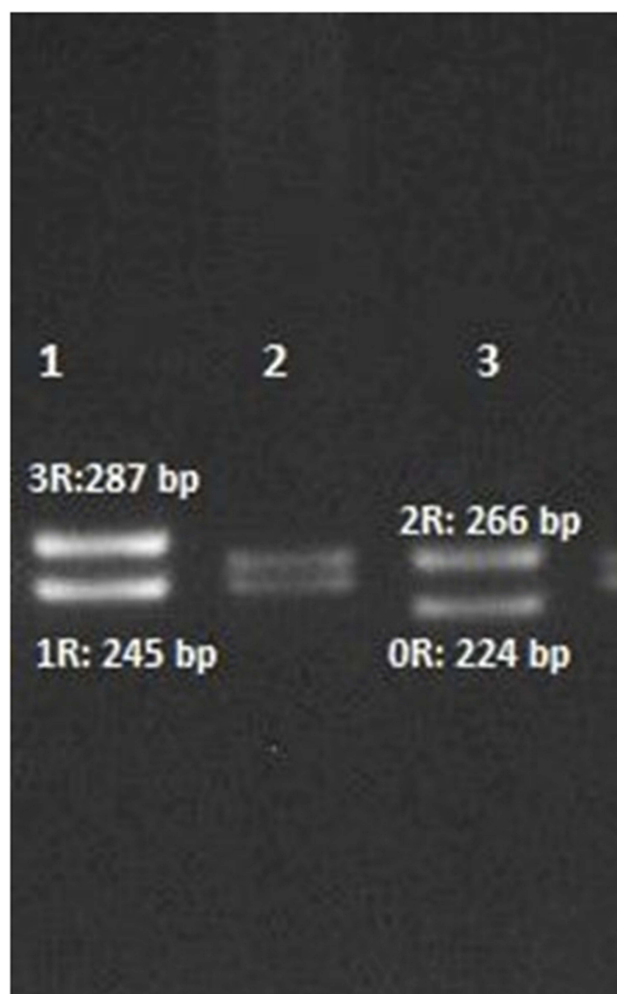


Figure 1 Agarose gel electrophoresis of the four alleles of the *XRCC5* promoter VNTR polymorphism after PCR amplification. Lanes 1, 2 and 3 show the 3R/1R, 2R/1R and 2R/0R genotypes, respectively.

genotype has the highest frequency among controls (71%) while most of the cases had the 1R\2R genotype.

Comparing between case and control genotypes (Table 1), a variation in genotypic frequencies was found between healthy women and BC patients. For instance, 35.5% of healthy subjects had the 2R\2R genotype compared to 19% of BC patients (P -value<0.0001). Moreover, there was a conspicuous disparity in 3R\3R genotype distribution among controls (1%) and patients (8.5%) with P -value<0.0001. Furthermore, significant association of genotypic frequency of 3R\2R genotype among cases and controls with BC risk was observed (P -value<0.0001). The estimated overall P -value for an association between *XRCC5* VNTR polymorphism and BC was<0.0001. In terms of the allelic frequency distributions of *XRCC5* VNTR polymorphism, a significant difference was observed among cases and controls. Our

findings showed a significant association between allelic frequencies of 3R and 2R in the study cohort (P -values<0.0001 and<0.001, respectively).

In addition, we investigated the correlation between certain clinical and pathological parameters of BC patients and the *XRCC5* variant. For each parameter, we compared between the groups of patients with different genotypes. Table 2 reviews patients' clinical characteristics compared with different *XRCC5* VNTR genotypes. These parameters include body mass index (BMI), age at first breast cancer diagnosis, age at first pregnancy, age at menarche, age at menopause, co-morbidity, allergies and lifestyle habits such as smoking. In this study, we did not find any correlation between these investigated criteria among BC patients and the VNTR of *XRCC5* gene. In addition to clinical features, the existence of any link between pathological features and BC risk was explored. Table 3 summarizes the studied pathological parameters, which included progesterone and estrogen receptors status (PR and ER), human epidermal growth factor receptor 2 (Her2) marker, extent of tumor differentiation (low vs middle and high), tumor size, lymph node involvement and others. In this study, BC was classified into in situ carcinoma and invasive carcinoma. Based on the genetic analysis, we found that 18.9% of patients were diagnosed with in situ carcinoma (2R and/or 3R; 27.8%, 0R and/or 1R; 72.2%) while invasive carcinoma accounted for 81.1% (2R and/or 3R; 35%, 0R and/or 1R; 65.0%).

In addition, the genotypes of *XRCC5* VNTR were investigated for correlation with different types of BC according to the immunohistochemistry (IHC) profiles of BC patients, these types categorized according to the presence or absence of ER, PR, and HER2 markers (Table 3). However, our results did not show an association between the pathological parameters and *XRCC5* VNTR.

Discussion

Breast cancer (BC) comprises a large part of the disease and injury burden for women and poses a direct threat to their health and overall welfare.^{20,21} From among BC risk factors, the role of genetics in BC development has been widely investigated, and numerous genetic polymorphisms suggested to influence its progression have been screened.^{22,23} Different genotypes of the VNTR in the *XRCC5* gene have been identified by several early studies.^{24,25} In the present study, ten different genotypes and four alleles of the *XRCC5* VNTR polymorphism in its promoter region were assessed for any relation with BC risk in Jordanian females.

Table 1 Genotype and allele frequencies of the *XRCC5* VNTR in breast cancer patients and healthy individuals

Genotypes/alleles	Controls (n=200); n (%)	Cases (n=200); n (%)	P-value* cases vs controls	χ^2	Overall P-value*
0R\0R	2 (1%)	0 (-)	NA	42.4	<0.0001
1R\0R	7 (3.5%)	5 (2.5%)	0.42		
1R\1R	50 (25%)	45 (22.5%)	0.69		
1R\2R	56 (28%)	52 (26%)	0.69		
2R\0R	6 (3%)	9 (4.5%)	0.61		
2R\2R	71 (35.5%)	38 (19%)	<0.0001		
1R\3R	0 (-)	3 (1.5%)	NA		
3R\2R	6 (3%)	31 (15.5%)	<0.0001		
3R\3R	2 (1%)	17 (8.5%)	<0.0001		
0R	17 (4.25%)	14 (3.5%)	0.54	48.8	<0.0001
1R	163 (40.75%)	150 (37.5%)	0.95		
2R	210 (52.5%)	168 (42%)	<0.001		
3R	10 (2.5%)	68 (17%)	<0.0001		

Notes: *P-value<0.05 is considered significant. χ^2 : Chi square.

Abbreviation: NA, not applicable.

Table 2 Association of the *XRCC5* VNTR polymorphism with the clinical characteristics of breast cancer patients

Clinical characteristics	P-value
BMI**	0.238
First pregnancy (age)**	0.785
Age at breast cancer diagnosis**	0.462
Age at first menstruation**	0.786
Breastfeeding status*	0.172
Age at menopause**	0.448
Family history*	0.817
Allergy*	0.091
Smoking*	0.586
Co-morbidity*	0.345

Notes: *Genotype-phenotype association P-value using Pearson Chi-Square test.

**Genotype-phenotype association P-value using ANOVA test. P-value<0.05 is considered significant.

XRCC5 gene encodes the Ku80 heterodimer subunit. The Ku80 acts in non-homologous end joining (NHEJ) pathway by binding to DNA double-strand break ends. In addition, this heterodimer subunit plays a key role in homologous recombination (HR). A (VNTR) insertion in *XRCC5* promoter region can influence its expression and affect Ku80 synthesis. As a consequence, these changes may affect the NHEJ and HR pathways leading to multiple types of sporadic cancer including BC.¹⁴

Previous studies claimed that the VNTR in the *XRCC5* gene is vary among the ethnic group.¹⁸ Cui et al,¹⁴ compared the frequency of all VNTR genotypes among 100 healthy local Caucasian individuals with the genotypes from 535 Caucasian Iranian and 235 Chinese populations. The incidence of 2R\2R genotype in our finding was

35.5% which is far from it in Caucasian (16%), Iranian (2%) and Chinese (5%). Whereas the frequency of homozygous 1R allele among healthy subjects in this study (25%) was compatible with it among Caucasian (22%) and in contrast to it among Iranian and Chinese populations (5% and 2%), respectively. Additionally, the 0R\0R genotype was similar to it among these three population. According to Cui et al,¹⁴ the 3R\3R genotype was not detected among Caucasian, Iranian and Chinese. However, only 1% of 3R\3R genotype was detected among healthy individuals in the current Jordanian study. Our results suggest that the genotypic distribution among BC patients and healthy controls was significantly linked to breast cancer (P -value=1.5e-7). A significant difference in the distribution of 2R\2R, 2R\3R and 3R\3R genotypes between cases and controls, proposing that the 2R and 3R may be in association with BC. In the current study, we also observed that the frequencies of the genotypes with low number repeats (0R and 1R) were higher than the genotypes with high number repeats (2R and 3R) among BC cohort which contrasts with the *XRCC5* VNTR polymorphism distribution among controls. Depending on the statistically significant results, the distribution of 2R\2R genotype was higher than 3R\3R genotype distribution frequency among BC patients. Furthermore, 2R allele was more frequent than the 3R allele among BC patients. As a consequence, this study suggested that 2R alleles may influence BC increased risk.

Several studies demonstrated the role of *XRCC5* VNTR polymorphism in cancer development and progression.

Table 3 Association of *XRCC5* VNTR polymorphism with pathological characteristics of BC patients

Pathological characteristics	P-value
Progesterone receptor*	0.313
Estrogen receptor*	0.396
Human epidermal growth factor receptor 2 (Her2) marker*	0.603
IHC profile*	0.324
LA vs LB vs TN	
Tumor differentiation*	0.638
Axillary lymph nodes*	0.821
Tumor stage*	0.130
Histology classification*	0.589
Tumor size**	0.288
Lymph node involvement*	0.967

Notes: *Genotype-phenotype association P-value using Pearson Chi-Square test.

**Genotype-phenotype association P-value using ANOVA test. P-value<0.05 is considered significant. IHC: the immunohistochemistry (IHC) profile: LA: ER(+) and/or PR(+) plus HER(-). LB: ER(+) and/or PR(+) plus HER(+). TN: ER(-) and PR(-) plus HER(-).

Rajaei et al,¹⁸ reported that the 3R allele of the *XRCC5* VNTR leads to reduced gene expression. Since *XRCC5* overexpression has been linked to cancer, an increase in the *XRCC5* VNTR repeat number may confer protection against cancer. Subsequent research by Rajaei et al,²⁶ determined that the 0R\0R genotype increased BC risk compared to the 1R\1R genotype, while the 3R\1R genotype decreased BC risk compared to the 1R\1R genotype.

In addition, a group of clinical and pathological criteria thought to be BC risk factors were investigated and statistically tested for any significant association with *XRCC5* VNTR. However, we failed to detect any correlation between BC prognosis factors including clinical and pathological features and *XRCC5* VNTR polymorphism.

To conclude, the *XRCC5* VNTR polymorphism is strongly associated with BC among Jordanian women but not with the clinicopathological parameters. More epidemiological studies are recommended to clarify the underlying role of *XRCC5* genetic variant among different ethnic groups.

Ethical approval and informed consent

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Review Board (IRB) at Jordan University of Science and Technology with ethical code number 32/104/2017. Written informed consent was obtained from all individual participants included in the study.

Data availability

The data sets generated and/or analyzed over the course of the study are not publicly available but are available from the corresponding author on reasonable request.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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